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SIDNEY RINGER

THE

COLLECTED PAPERS

OF

SYDNEY RINGER,

M.D., F.R.C.P., F.R.S.

Vol. I.—Papers contributed to the "Journal of Physiology."
Vol. II.—Papers contributed to "The Practitioner."
Vol. III.—Contributions to various Journals.

ALSO

A List of the Papers and References contained in
"The Lancet," and "British Medical Journal,"

AND

An Appreciation of Dr. Ringer,
by Benjamin Moore, M.A., D.Sc., M.R.C.S., L.R.C.P.

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CONTENTS.

VOLUME I.

PAGE

In Memory of Sydney Ringer, 1835-1910. By Benjamin Moore . 1

Concerning the Effects on Frogs of Arrest of the Circulation, and an Explanation of the Action of Potash Salts on the Animal Body. By Ringer and Murrell 22

The Action of Arseniate of Soda and Arsenious Acid on Frogs. By Ringer and Murrell 47

Concerning the Action of Aconitia on the Nervous and Muscular System of Frogs. By Ringer and Murrell 66

The Action of Tartar Emetic, Hydrocyanic Acid and Veratria on the Animal Body. By Ringer and Murrell 75

On Pituri. By Ringer and Murrell 81

A Few Further Experiments with Pituria. By Ringer and Murrell. 90

On the Antagonism between Pilocarpine and Extract of Amanita Muscaria. By Ringer and Murrell 93

On the Physiological Action of Narcissia, an Alkaloid, obtained from the Bulb of the common Daffodil (Narcissus Pseudo-Narcissus). By Ringer and Morshead 103

On the Physiological Action of the common Snowdrop (Galanthus Nivalis) and of the Jonquil. By Ringer and Morshead . . 123

On the Relative Paralysing Action of Atropia and Pilocarpine on the Heart. By Ringer and Morshead 127

Concerning the Action of Common Salt, Sulphate of Atropia, Bromide of Conia and Sulphate of Nicotine on Muscular Irritability. By Ringer and Morshead 144

On the Antagonisms of Aconitia on the Frog's Heart 154

Concerning the Influence of Season and of Temperature on the Action and on the Antagonisms of Drugs 161

Regarding the Action of Hydrate of Soda, Hydrate of Ammonia, and Hydrate of Potash on the Ventricle of the Frog's Heart . 171

Concerning the Influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricle 179

PAGE

A Further Contribution Regarding the Influence of the Different Constituents of the Blood on the Contraction of the Heart . 193

A Third Contribution Regarding the Influence of the Inorganic Constituents of the Blood on the Ventricular Contraction . 207

Of the Influence of certain Drugs on the Period of Diminished Excitability. By Ringer and Sainsbury 212

An Investigation Regarding the Action of Rubidium and Cæsium Salts compared with the Action of Potassium Salts on the Ventricle of the Frog's Heart 228

Concerning the Influence of Saline Media on Fish, etc. . . . 240

On the Mutual Antagonism between Lime and Potash Salts, in Toxic Doses 259

An Experimental Investigation showing that Veratria is similar to Lime Salts, in many respects, as regards their Action on the Ventricle, also showing that Veratria and Potash Salts are reciprocally Antagonistic 267

Concerning the Action of Small Quantities of Calcium, Sodium, and Potassium Salts upon the Vitality and Function of Contractile Tissue and the Cuticular Cells of Fishes. By Ringer and Buxton 275

An Experimental Investigation to ascertain the Action of Veratria on a Cardiac Contraction 283

Regarding the Influence of the Organic Constituents of the Blood on the Contractility of the Ventricle 287

A Further Contribution regarding the Effect of Minute Quantities of Inorganic Salts on Organised Structures 310

Further Experiments regarding the Influence of Small Quantities of Lime, Potassium and other Salts on Muscular Tissue . . 321

Regarding the Action of Lime, Potassium and Sodium Salts on Skeletal Muscle 340

Concerning Experiments to Test the Influence of Lime, Sodium and Potassium Salts on the Development of Ova and Growth of Tadpoles 345

Concerning the Action of Calcium, Potassium and Sodium Salts upon the Eel's Heart and upon the Skeletal Muscles of the Frog. By Ringer and Buxton 351

Upon the Similarity and Dissimilarity of the Behaviour of Cardiac and Skeletal Muscle when brought into relation with Solutions containing Sodium, Calcium and Potassium Salts. By Ringer and Buxton 357

PAGE
Regarding the Action of Lime Salts on Caseine and on Milk . . 366
Further Observations on the Behaviour of Caseinogen . . . 382
The Influence of Calcium Chloride on Egg Albumen and some of its Derivatives 390
The Influence of certain Salts upon the act of Clotting. By Ringer and Sainsbury 403
The Action of Salts upon Heat Coagulation. By Ringer and Sainsbury 420
Further Observations on the Influence of Calcium Salts in Promoting Heat Coagulation of Albumens 436
The Influence of Carbonic Acid dissolved in Saline Solutions on the Ventricle of the Frog's Heart 445
The Action of Potassium, Sodium and Calcium Salts on Tubifex Rivulorum. By Ringer and Sainsbury 451
The Influence of Saline Media on the Tadpole. By Ringer and Phear 461
The Influence of Saline Media on Tubifex Rivulorum. By Ringer and Phear 471
Further Observations regarding the Antagonism between Calcium Salts and Sodium, Potassium and Ammonium Salts . . . 477
The Action of Distilled Water on Tubifex 483
List of Papers and References in *The Lancet* 485
List of Papers and References in *The British Medical Journal*. . 489

CONTENTS.

VOLUME II.

PAGE

On the Employment of Glycerine of Tannin 1

On the Employment of Digitalis in Diseases of the Heart . . 6

Purpuric Spots Produced by Iodides of Potassium and Iodide of Ammonium but not by Iodide of Sodium 19

On the Influence of Belladonna on Sweating 21

A Curious Case of Facial Neuralgia with Unilateral Sweating Cured by the application of Aconite Liniment 26

Some Additional Observations on the Action of Atropia on Sweating 30

A Case of Rheumatic Fever with High Temperature successfully Treated with Cold Baths 32

A Case of Rheumatic Fever Treated with a Cold Bath; Death occurring immediately on leaving the Bath 39

Notes of Five Cases of Paracentesis Thoracis 42

A Successful Case of Traumatic Tetanus Treated by Large Doses of Calabar Bean 51

The Effects of Pilocarpine on Two Cases of Unilateral Sweating . 61

A Case of Acute Mania Treated with Large Doses of Hyoscyamia, Daturine, Atropia and Ethyl-Atropia. By Ringer and Bury . 67

Notes on a Case of Athetosis, preceded by Hemiplegia and Hemianaesthesia, and accompanied by Unilateral Sweating . . 71

Notes of a Post-Mortem Examination on a Case of Athetosis . . 91

On the Relative Action of Duboisia and Atropia 107

On the Physiological Action of an Alkaloid Extracted from the Garden Tulip—Natural Order Liliaceæ 111

On the Action of Extract of Muscaria, of Nitrate of Pilocarpine, and of Extract of Jaborandi on the Ventricle of the Frog's Heart and on the Antagonising Action of Atropia 115

Influence of Anæsthetics on the Frog's Heart 124

PAGE

Concerning the Action of Chloride of Sodium, Chloride of Ammonium, Chloride of Potassium, Bromide of Sodium, Bromide of Ammonium, Bromide of Potassium, on the Ventricle of the Frog's Heart 143

On the Individuality of Action of the Component Parts of a Drug. By Ringer and Sainsbury 157

On the Antagonism between Veratria and Potassium Salts . . 171

Observations on the Relative Effects of Certain Members of the Ethylic Alcohol Series on the Ventricle of the Frog's Heart. By Ringer and Sainsbury 175

An Investigation regarding the Action of Strontium and Barium Salts compared with the Action of Lime on the Ventricle of the Frog's Heart. By Ringer and Sainsbury 188

An Experimental Investigation to ascertain in what manner Soluble Oxalates Arrest Function and some Remarks on the Action of Poisons and their Antidotes 202

List of Papers and References in *The Lancet* 211

List of Papers and References in *The British Medical Journal* . 215

CONTENTS.

VOLUME III.

Medico-Chirurgical Transactions.

PAGE

On the Connexion Between the Heat of the Body and the Excreted amounts of Urea, Chloride of Sodium, and Urinary Water during a Fit of Ague 1

Proceedings of the Royal Society.

On the Alteration of the Pitch of Sound by Conduction through Different Media 42

Medico-Chirurgical Transactions.

On the Relative Amounts of Sugar and Urea in the Urine in Diabetes Mellitus 47

On the Temperature, Urea, Chloride of Sodium and Urinary Water in Scarlet Fever, and on a Cycle in Disease and Health . . 77

Observations on Box (Buxus Sempervirens) with especial Reference to the True Nature of Tetanus. By Ringer and Murrell . . 133

Proceedings of the Royal Society.

On the Temperature of the Human Body in Health. By Ringer and Stuart 165

Journal of Anatomy and Physiology.

On the Effects of Sulphate of Atropia on the Nervous System of Frogs. By Ringer and Murrell 191

Further Observations and Experiments regarding the True Nature of Tetanus. By Ringer and Murrell 203

The Influence of Salicine on the Healthy Body, with Special Reference to its influence on the Temperature. By Ringer and Bury 215

Concerning the Action of Chloride of Potassium on the Nervous System of Frogs. By Ringer and Murrell 232

The Influence on the Afferent Nerves of the Frog's Leg from the Local Application of the Chlorides, Bromides and Iodides of Potassium, Ammonium and Sodium. By Ringer and Morshead 236

On the Effect of the Chlorides, Bromides and Iodides of Potassium and Sodium on Frogs. By Ringer and Morshead . . . 251

Archives of Medicine.

PAGE

On the Physiological Action of the Bulb of the Buphane Toxicaria or Hemanthus Toxicarius Amaryllidaceæ. By Ringer and Morshead 263

The Action of Veratria on the Ventricle of the Frog's Heart . . 283

Medico-Chirurgical Transactions.

Concerning the Action of Salts of Potash, Soda and Ammonia on the Frog's Heart. By Ringer and Sainsbury 293

Investigations into the Action of the Digitalis Group. By Ringer and Sainsbury 327

List of Papers and References in *The Lancet* 351

List of Papers and References in *The British Medical Journal* . 355

IN MEMORY OF SIDNEY RINGER

[1835-1910]

SOME ACCOUNT OF THE FUNDAMENTAL DISCOVERIES OF THE GREAT PIONEER OF THE BIO-CHEMISTRY OF CRYSTALLO-COLLOIDS IN LIVING CELLS

It is often notable, on consulting the original writings of master-workers in any domain of knowledge, to what an extent these contain, crystallized out in single sentences, or short paragraphs, discoveries of great beauty or intense importance, which have afterwards been re-discovered and related at ream's length by subsequent writers.

This observation will strike anyone who cares to dive into the original papers of Thomas Graham on colloids, and it is no less striking in the writings of his colleague, Sidney Ringer, on the correlated subject of the crystallo-colloids and the balanced activities of the inorganic constituents in regulating and controlling the energy discharges of living cells.

Both physical chemists and bio-chemists have nowadays come to realise that the most fruitful ground of both chemistry and biology lies in the land of colloids, and of colloidal and crystalloidal relationships. It is a remarkable fact that the master-discoveries in these two closely related domains belong to two men identified with the same institution, who, keen though they were over their experiments, dimly, if at all, realised the vast extensions which lay before their discoveries. Graham does appear to have dimly seen that there was a relationship between the energetics of the colloidal state and the life processes of cells, and draws attention to the fact that colloids are unstable and all the time suffering slow energy changes, while crystalloids are inert and inactive in stable equilibrium.

Ringer equally appreciated the importance of the inorganic salts in living processes, in stimulating or stilling muscular activity, or in varying the velocity of growth and repair in living cells of all types.

But the state of advancement of knowledge at the time the discoveries were made precluded either observer from surveying the whole field and seeing how much all these researches were complemental to one another, and that the ultimate substrate for the energy display was the delicately balanced equilibrium between colloid and crystalloid.

Ringer's researches, which yield such a magnificent demonstration of the applicability of the fundamental principles of the ionic theory, were made independently of it, and before it had gained any general acceptance. Accordingly, one can only marvel now at the experimental inspiration and clear logical following out of sequences which enabled him to develop one fact after another, track out one possibility after another, and eliminate everything extraneous until he had found the true connection between the phenomena with which he was dealing.

Remarkable accuracy in observing detail, thoroughness in investigating every factor in the problem, however trivial at first sight that factor might seem, and untiring energy in repetition of experiment until he was quite certain he held the whole truth of the case without omission or exaggeration, were the traits which went to make up Ringer's character, both as a scientific observer in the laboratory and as a clinician in the hospital. At the active period of his scientific work he was a most prolific writer, yet all his work, so far as he was responsible, has stood the test of time and grown in beauty in men's eyes with the lapse of the years.

In one of his later conceptions only was Ringer misled by a circumstance over which he had no control. The laboratory supply of distilled water contained an unsuspected impurity, viz., a trace of copper, due to a defect in the still, and this misled Ringer as to the action of the supposed pure distilled water upon living organisms. The writer happened at the time to be working in collaboration with him at another piece of work, and, at his request, tested the laboratory water: he can still well remember Ringer's chagrin and disappointment when the presence of copper in the water was demonstrated to him. This incident, although he could not possibly have foreseen it, troubled him not a little; he temporarily ceased work upon his beloved subject, and other events which meanwhile occurred in private life conspired to prevent him ever afterwards taking up laboratory work with his wonted energy, so that he never contributed further to the subject, with the exception of a short note to the Physiological Society, published about two years later.[1]

The first of the remarkable series of papers which record Ringer's discoveries of the actions of inorganic salts (or ions) upon the beat of the heart, is contained in the third volume of the *Journal of Physiology*, 1881-2, under the title 'Concerning the influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricle.' This first paper demonstrates clearly Ringer's scientific insight and doggedness of purpose. He shows that certain changes in the ventricular beat, which, at this stage, he thinks are due to normal saline (0·75 per cent. solution of sodium chloride), but which in a later paper he finds out are due to a trace of calcium salt in the supply of New River Water with which the saline has been made up—he shows that these changes can be obviated if a certain amount of dried blood is added to the saline. Proceeding further, he finds that diluted white-of-egg added to the saline produces a like result in maintaining the normal heart-beat. The ordinary investigator would probably have stopped here, thinking he had done well and probed matters to the limit. Not so Ringer, and this is precisely how he made the first step that counts so much towards his great discovery. Having discovered that both dried blood extract and diluted egg-white act upon the contracting ventricle, he does not jump to what, at that stage of our knowledge, would be the most obvious conclusion, viz., that this common good result arose from the nutrition of the contracting ventricle by the proteins common to both fluids. Here are the discoverer's own words on the subject:—'Blood, then, obviates the changes occurring in the contractions of a ventricle receiving only saline solution, and the question arises, which constituent of the blood has this property?'

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[1] This note takes up the matter of the supposed action of distilled water on Tubifex, and is characteristic of Ringer, both in its carefulness and exactitude and in the candour and honesty with which it admits the truth of Locke's position : that the supposed poisonous and disintegrating action of distilled water obtained by distillation from a metal still and condenser are due to *infinitesimal* traces of heavy metals in the water. He first of all re-distilled from glass thirty litres of the type of water used originally with poisonous effects, and now found the distillate harmless, and the residue to contain copper and be violently poisonous until after exposure to air for some days. Then he collected Nature's own distillate in the form of rainwater on his Yorkshire moorside in earthenware vessels, and demonstrated that this water was harmless to the organisms although it contained no trace of calcium salts. On placing a few small pieces of copper filings in this innocuous water it became poisonous and disintegrated Tubifex in a few hours. As a result of this careful enquiry he frankly admits that : 'These experiments appear to me to establish Locke's conclusion that copper in even infinitesimal quantities will disintegrate Tubifex, whilst water free from copper or other heavy metals, and without any salts such as calcium salts, can sustain the life of Tubifex.' In spite of this disappointment it is interesting to observe Ringer's mental activity in seizing the opportunity to demonstrate to the hilt the most important fact which he had earlier observed as to the antagonism of the potassium and calcium effects. He says, 'I next tested with this rainwater from the moors the antagonism between potassium chloride and calcium chloride. I placed Tubifex in a 0·1 per cent. solution of potassium chloride, in about four hours they were almost motionless, and with the microscope I was unable to see the vessels contract; they appeared quite paralysed except just below the head of the animals. I then placed the Tubifex in a 0·1 per cent. KCl and 0·1 per cent. $CaCl_2$ and almost at once spontaneous movement increased, and in five minutes they became quite active. Seven hours later they were moving energetically; before movement was so much restored the microscope showed that the vessels, even to the tail were contracting vigorously.' (*Proc. Physiol. Soc.*, December, 1897, *Journ. of Physiol.*, Vol. XXI, pp. 14, 15.)

'I find that a small quantity of white-of-egg completely obviates the changes occurring with saline solution. I first took tracings with blood mixture; I then replaced the blood with 100 c.c. of saline, and got the usual great prolongation of dilatation. I then added to the saline 25 c.c. of white-of-egg mixture, composed of one part white-of-egg in two parts of water. In two minutes the contractions became exactly as they were when the heart was supplied with blood mixture. White-of-egg consists of albumins and chloride of potassium and sodium, chiefly chloride of potassium. It is obvious in the preceding experiment that the effect of white-of-egg could not be due to the sodium chloride. The power to obviate the great prolongation of the dilatation occurring when the ventricle was supplied with saline solution must, then, be due to the albumins or the potassium chloride.'

'I find that potassium chloride in small quantities, much smaller than exists in serum, will completely and speedily obviate the character of the trace occurring with saline solution, and give a trace in all respects like that occurring when the ventricle is supplied with blood mixture.' [References to the tracings here.] 'I first took a tracing with blood mixture, and then replaced the blood with 100 c.c. of saline solution. After about 15 minutes I obtained the usual great prolongation of the ventricular dilatation. I then added to the 100 c.c. of circulating saline 0·7 c.c. of 1 per cent. solution of potassium chloride at the point indicated by the arrow; very speedily the contraction became modified, the dilatation becoming much shorter. Ten minutes after the addition of the potassium chloride the contraction became just like those at the beginning of the experiment when the ventricle was supplied with blood. From numerous experiments, I find that from 0·6 c.c. to 1 c.c. of 1 per cent. solution of potassium chloride to the 100 c.c. of saline solution is sufficient to remove the prolongation of dilatation occurring with saline solution.'

At the date of this paper the ionization theory of Arrhenius had not yet appeared on the scientific horizon, and hence it is most interesting to observe Ringer, in complete ignorance of it of course, proceeding to accumulate evidence in strong support of it had it only been known later to the supporters of the theory in their days of battle.

In the same manner, as recounted in his own words above, Ringer proceeded to test the actions of phosphate of potash and phosphate of soda (as he calls them), and found that phosphate of potash had precisely the same action as potassium chloride, while phosphate of soda had no action. His conclusion is that, 'the effect, then, in potassium phosphate is due to the potassium and not to the phosphoric acid.'

'Probably any potassium salt is sufficient to obviate the prolonged dilatation occurring when the ventricle is fed with saline solution only, for I have produced normal contractions by adding to the saline solution potassium chloride, potassium phosphate, potassium sulphate, potassium citrate, potassium bi-carbonate, potassium chlorate, potassium nitrate, potassium acetate, and potassium carbonate.'

Throughout this paper Ringer is ignorant of the fact that the prolongation of ventricular relaxation antagonized by the potassium salts is due to the presence of an unbalanced trace of calcium salt in his saline solution. It is a remarkable piece of scientific good fortune that this accident had occurred, for although the diminution in force of beat and stoppage in pure distilled water *plus* sodium chloride is characteristic enough as compared with the full 'Ringer's effect' as now known to us, yet any such slowing down without the long tailing off produced by unbalanced calcium ion would probably not have attracted Ringer's attention in these earlier days, or called forth his energies to the same extent.

In his next paper, however, in the fourth volume of the *Journal of Physiology*,[1] he has discovered the great influence of minute traces of calcium upon the heart. Previous work of his own and of others upon the antagonistic action upon the heart of different alkaloids had prepared his mind, and the thought of an antagonism, as he calls it, between calcium and potassium fell upon prepared ground. In this second paper he clearly shows how different inorganic constituents can balance or antagonise one another, and this is reiterated in paper after paper of the succeeding members of the long series, and upon one type of material after another, such as ventricular muscle, skeletal muscle, growing frog's eggs, tadpoles, small fishes, tubifex worms, and so on. It is indeed remarkable that any man should have so reiterated and driven home a discovery of such first-rate importance, himself recognising its great value, although naturally he did not realise its more modern extensions, and that then for years he should be almost unknown in connection with it, save perhaps as the inventor of 'Ringer's solution.'

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[1] The papers are not dated in these earlier volumes of the *Journal of Physiology*, and it is somewhat difficult to accurately assign dates; the paper immediately preceding Ringer's is dated November, 1882, so the date must be the end of 1882, or early in 1883. A later reference given by Ringer himself in Vol. XVI of the *Journal of Physiology* places these earlier papers in 1883 (See *Journ. Physiol.*, Vol. XVI, p. 1).

The description, in the discoverer's own words, of how he came upon the calcium effect is highly interesting. He says:—'After the publication of a paper in the *Journal of Physiology*, Vol. III, No. 5, entitled "Concerning the influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricle," I discovered that the saline solution which I had used had not been prepared with distilled water, but with pipe water supplied by the New River Water Company. As this water contains minute traces of various inorganic substances, I at once tested the action of saline solution made with distilled water, and I found that I did not get the effects described in the paper referred to. It is obvious, therefore, that the effects I had obtained are due to some of the inorganic constituents of the pipe water.'

He then quotes a detailed analysis of New River Water, showing that amongst other things it contains 38·3 parts in a million of calcium, and proceeds experimentally to investigate calcium effects upon the ventricle. He shows that saline made from distilled water with pure sodium chloride does not give the rounded top and slow tailing off, but only shows a gradual cessation of activity altogether; then he adds a trace of calcium salt and obtains the now well-known result previously given by the New River Water saline. Further addition of the necessary small amount of a potassium salt takes away the slow relaxation and gives a normal beat which does not diminish and cease like that in presence of sodium chloride only, but remains strong and good for hours. After much experimentation and elimination or due appraising of actions of different constituents, Ringer describes the separate and combined effects as follows:—

'The heart's contractility cannot be sustained by saline solution, nor by saline containing potassium chloride, nor with saline solution containing bicarbonate of soda, nor by saline solution containing bicarbonate of soda and potassium chloride; but after contractility has ceased, the addition of a lime salt will restore good contractility. The addition, too, of a calcium salt to any of the above solutions will sustain contractility. I conclude, therefore, that a lime salt is necessary for the maintenance of muscular contractility.' 'But while calcium salts are necessary for the proper contraction of the heart, yet if unantagonised by potassium salts the beats would become so broad and the diastolic dilatation so prolonged that much fusion of the beats would occur and the ventricle would be thrown into a state of tetanus.' 'As the ventricle will continue to beat perfectly for hours without any sodium bicarbonate, it is evident that the normal trace is the result of the antagonising action of calcium and potassium salts.' 'If these two salts [we should say ions at the present day] are not present in the correct proportions, then the trace becomes abnormal. If too little potassium is present, the contractions become broader, etc., and there results fusion of the beats. If too much potassium is present, or too little lime salts, then the contraction of the ventricle is imperfect, and by increasing the quantity of potassium salt the beat becomes weaker and weaker till it stops.' 'As the heart will continue to beat quite normally for hours without albumen or haemoglobin, it is obvious that these substances are not immediately necessary for contraction, but of course they are necessary to reconstruct the tissues from the loss due to contractions.'

In these few sentences we have the whole law and gospel of the action of inorganic constituents on contractile tissue, and since then there has been chiefly adding of detail. A great deal of detail was added in this and later papers by Ringer himself, and he demonstrated the same relationship in the excitation of rhythmic contractions in skeletal muscle and their inhibition by the antagonistic salts. In the next paper Ringer tests the effects of an excess of potassium salts, and finds that the latent period is greatly prolonged, and also the period of diminished excitability, so that the frequency of beat becomes diminished. In this paper also he points out a remarkable change to faradic stimulation of the ventricle induced by potassium. In absence of any excess of potassium, faradization causes a fusion of beats in a contracted or systolic condition which Ringer, following Marey, speaks of as tetanus; in presence of an excess of potassium there is a reverse action, for now faradization completely arrests the contractions which recommence on discontinuing the faradization.

Rubidium in all respects acts similarly to potassium, but the higher member of the alkali group, viz., caesium, possesses a quite different action.

'Caesium, indeed, produces for the most part the opposite effects to potassium, broadening the trace, rounding its top, inducing fusion; yet in spite of this great difference in its action, still caesium, like potassium, possesses the property of obviating the effect of calcium on the dilatation.' He finds that calcium and caesium make a fairly good balance; but strontium and caesium will not make a good working pair. Strontium and potassium work; but not barium and potassium. The separate action of caesium resembles that of barium, but barium and calcium do not balance as do caesium and calcium.

'It is interesting to compare the effect of chemical similarity on physiological similarity in the group calcium, strontium, and barium, and in the group including potassium, rubidium, and caesium.'

'Strontium is chemically more allied to calcium than barium is to calcium, and whilst strontium is in its physiological action very similar to calcium, barium is far less similar to calcium. So it is with the group potassium, rubidium, and caesium. Rubidium is chemically more allied than caesium to potassium, and rubidium is almost identical in its physiological action with potassium, whilst caesium differs in most of its physiological effects from potassium.'

The effects of saline media on fishes are discussed in a communication to the Physiological Society in December, 1883, and in a most interesting paper appearing a few months later in the *Journal of Physiology* (Vol. V, p. 98). In these researches much interesting matter is brought together as to the behaviour of both fresh-water and salt-water fish when subjected to variations in the salinity of the medium in which they are living. It is demonstrated by Ringer that even fresh-water fish, such as minnows and sticklebacks rapidly perish when kept in distilled water,[1] and the effects of variations in oxygen supply being ruled out by experiment, it is shown that sodium, potassium and calcium salts are necessary to life in water. He shows that calcium in small amounts greatly prolongs life in distilled water, and that additional increase of longevity is obtained when sodium carbonate and potassium chloride are also added.

The concentration of calcium necessary for the sustenance of life is very low, but this can be exceeded manifold without causing the death of the fish; thus, as much as 0·5 per cent. of calcium chloride in the water could be borne by minnows.

In the next paper Ringer proceeds to examine the effects upon heart muscle of large toxic doses of calcium and potassium in the unbalanced and balanced conditions, and shows that a dose of either salt (or ion) which would be many times sufficient to poison the ventricle can be borne without ill effects when balanced by its antagonist. The paper might rank as a study of a balanced reaction at the present day, now about twenty-six years later. The whole subject illustrates the balancing of toxin and anti-toxin, as shown with inorganic ions. The calcium and potassium ions do not unite in stochiometric relationships with one another; in fact they have no appreciable chemical affinity for each other; but both are claimants for the bio-plasm of the ventricular muscle-cells, and in proper proportions determined by their chemical coefficients for the bio-plasm, we get balancing and the to-and-fro beat of the heart. This ratio can evidently exist at many corresponding concentrations of potassium and calcium, but a balance must be maintained. To each concentration of either of the two, there is a short range within which the other ion must lie.

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[1] In view of later discoveries it would now be of interest to repeat these earlier experiments of Ringer on the action of distilled water on organisms.

The next paper, also in the fifth volume of the *Journal of Physiology*, demonstrates that veratria behaves similarly to calcium upon the heart ventricle, and can even replace lime salts, though somewhat imperfectly. The interesting fact is also shown that veratria and potassium, just like calcium and potassium, are mutually antagonistic.

In a second paper, dealing also with veratria and potassium, Ringer attempts some analysis of the different molecular reactions, as he terms them, occurring during contraction, relaxation, and reparation in the ventricular tissue. His words show a remarkable insight for the period at which they were written, and historically are worthy of quotation.

'In a muscular contraction three distinct forms of molecular change are obvious; namely, the changes occurring during the latent period, the changes which occur during contraction, and the changes causing the dilatation.' 'Those molecular changes which lead to dilatation are generally considered restorative or reparative; they prepare new material for the ensuing contraction, or remove waste products which would retard or prevent contraction; or they consist of both of these processes combined.' 'The independence of those molecular changes which occur at the termination of a contraction (and which cause dilatation) from the reparative changes is well shown by the action of lime on the ventricle. Calcium chloride, added to saline solution, greatly delays dilatation, but does not in any degree delay the reparative changes, so that no matter at what period the excitation is made, the succeeding contraction is complete, even though the excitation is applied before dilatation is finished. Here, then, we have a drug which, like veratria, retards those molecular changes that lead to dilatation, but, unlike veratria, in no degree lessens the rapidity of the reparative changes.'

'We have seen that both veratria and potash salts retard the reparative changes, veratria more so than potash, and yet when these substances are both added to the circulating fluid they do not in this respect intensify each other's action, but distinctly antagonise each other, the reparative changes occurring much more rapidly after the addition of potash to the veratrized circulating fluid. We have, then, another instance of drugs having a common action, and yet when both are conjointly added to the circulating fluid, one antagonises the other in respect of this common effect.'

In a somewhat lengthy paper entering into a discussion of contemporary continental opinion as to whether organic nutrition is directly necessary to the heart-beat, Ringer attempts to separate all salts by dialysing albumin solutions, but finds it impossible to get rid of the calcium which adheres after prolonged dialysis. He then shrewdly suspects what we now know to be the correct view, that it is this strongly adherent calcium which enables albumin solutions to keep up contractions without the addition of inorganic calcium salt.

The next contribution is a most interesting one on the effect of lime salts upon the absorption of water by laminaria and other organic structures. It is full of interesting observations, the details of which cannot be entered upon here; but it is well worthy of perusal to-day by anyone interested in the modern problems of absorption of salts by colloids.

In a set of three papers following rapidly on this, Ringer demonstrates most clearly the action of calcium and other ions upon *skeletal* muscle. The first of the series is by Ringer alone, and in it the muscle is simply immersed in the various saline solutions. In a later paper, in collaboration with Dudley Buxton, the similar effects are described which are obtained when the various solutions are perfused from the aorta.

These papers are of great historic interest to all students of the action of inorganic ions on living cells, and since Ringer's connection with this work appears to have been well nigh forgotten, some recognition may here be made by quoting his main findings from the earlier paper, and reproducing certain of his tracings.[1] The paper is contained in the *Journal of Physiology,* Vol. VII, pp. 291-308, 1886. It is noteworthy that it was here that Ringer first commenced to use calcium phosphate in his circulating fluid, having previously used chlorides only of sodium, potassium and calcium, that is up till this stage, he had himself always used what is now a modern modification of Ringer's solution. It is also of interest that his calcium phosphate, as he tells us, was analysed for him by his colleague Dr. Graham. Professor of Chemistry at University College. After exhaustive experiments on the periods during which skeletal muscles kept in various saline combinations retained their direct faradic excitability, he classifies them in the following order and then proceeds to describe his experiments on the contractions of skeletal muscle in saline solutions:—

'We see then that these solutions in respect of their efficiency to sustain contractility stand in the following order. Saline solution is the least effective, next follows saline containing sodium bicarbonate, then phosphate of lime saline, next phosphate of lime saline containing potassium chloride. Phosphate of lime saline containing potassium chloride and phosphate of lime saline containing potassium chloride and sodium bicarbonate sustain contractility about an equal time. Phosphate of lime saline sustains contractility longer than saline solution containing sodium bicarbonate.'

'Now this being the order of their efficiency as circulating fluids for the heart, it would appear that lime and potash salts are as necessary to the metabolism of muscle at rest as to the metabolism occurring during a muscular contraction.

Another circumstance however explains in part the efficacy of lime and potassium salts in sustaining contractility in quiescent muscle.

I noticed in the muscles placed in saline active contractions. This was much more apparent in muscles that were partially detached, and especially noticeable in the sartorius when torn from the rest of the muscles and attached only by its lower end. Now these contractions must consume the energy of the muscles and hasten exhaustion and the advent of rigor mortis.

In February I commenced a further series of experiments regarding the effect of saline solution and saline solution containing other salts to produce contractions.

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[1] For permission to make these copious extracts, and to reproduce the accompanying traces, the thanks of the writer are due to Prof. J. N. Langley, the Editor of the *Journal of Physiology.*

Since I made these observations I find that Biedermann[1] has made similar experiments with results quite in accord with mine. Our methods however differed in some respects, and I recount here certain additional experiments to those he performed.

In his experiments he employed the sartorius of a strongly curarized frog. He suspended the muscle in a solution consisting of a litre of water, 5 grams NaCl, 2·5 grams Na_2HPO_4, and 0·4 gram Na_2CO_3, and in most of his experiments he kept the temperature below 10° C. He obtained contractions in all respects like those described in my experiments, but I employed all the muscles of the thigh cut into thin ribands and attached only at the knee. I did not curarize the frog and I placed the limb in simple saline. Biedermann took no tracings of the contractions.

It is obvious therefore that the alkaline soda salts do not play so important a part in the production of these contractions as Biedermann supposes. I shall show that phosphate of soda or caustic soda added to saline increases somewhat the frequency and the strength of the contractions. As I have said though working under different circumstances I obtain the same results, so well described by Biedermann.

We both find that temperature strongly influences these contractions, these being more frequent and more vigorous at a temperature between 20 and 30° C. than below 10° C. At a low temperature the contractions become somewhat rhythmical. There occur long pauses free from contractions, varying from ten seconds to two or three minutes, and then a series of contractions take place of much the same strength and separated by about the same intervals. Occasionally the beats for a minute or two are quite rhythmical.

I cut off both hind limbs of a frog through the hip joint and after stripping off the skin I cut the thigh muscles into thin ribands, and left them attached only at their lower end close to the knee joint. One limb I suspended in 200 c.c. ·6 per cent. saline solution, the other in a similar quantity of saline solution containing some other salt.

On placing the limb in saline solution the strips of muscles at once contract frequently, and one sees both massive and fibrillary contraction, i.e., the whole muscle or piece of muscle is contracted, and in addition one sees the contraction of fibrillae merely. The contractions begin soonest and are most marked in the thigh muscles cut into thin bands. Later fibrillary contractions occur in the uninjured lower leg muscles causing twitching of the toes. These contractions persist often for hours and continue longer in the uninjured than in the thigh muscles cut into ribands.

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[1] *Wien. Sitzungsbericht.* Bd. LXXXII, 1880, p. 257.

As Biedermann noticed with his solution so I find that when the contractions have ceased they often recur on renewing the saline solution.

On removing the limb from saline, strong fibrillary contractions persist for some time, and when they cease the excitation of a contraction by the faradic current brings them back again to persist without any further stimulation for half a minute or even longer.

The muscles of the fellow limb placed in phosphate of lime saline solution twitch much less, and in some cases no contractions at all occur.

The addition of 1 c.c. of 1 per cent. solution of potassium chloride to each 100 c.c. of the testing fluid very slightly lessens the fibrillary twitching of the muscles in saline solution. The twitchings of the muscles in phosphate of lime saline solution are greatly lessened or even utterly arrested; but on replacing the muscles in their original fluid the twitching again increases, and on the further addition of potassium chloride the same effect follows as on the first addition.'

'I find that small doses of potassium chloride 1 c.c. to 4 c.c. of 1 per cent. solution added to 100 c.c. of saline somewhat lessen the contractions. These quantities added to phosphate of lime saline solution at once check any movements, though, as I have said, phosphate of lime without the aid of potassium chloride generally prevents any movements.'

' Saline made with tap water prevents these twitchings, as the following experiment proves.

On Jan. 20 I placed one posterior extremity in 200 c.c. distilled water saline, the other in 200 c.c. tap water saline. The muscles in the distilled water [saline] began to twitch in three minutes and in ten minutes became quite brisk, the lower leg muscles quivering actively as well as the partially detached muscles of the thigh. The twitchings continued all day and were quite energetic eight hours from the beginning. The muscles in tap water saline did not twitch at all.

Contrary to what one would expect distilled water causes few or no twitchings.

On Jan. 20 I placed one hind limb of a frog in 200 c.c. distilled water saline and the other in distilled water. Active twitching persisted in the muscles in saline solution three hours, but had ceased in five hours and a half. In the muscles in distilled water one twitch occurred five minutes after the immersion but no other movement, and all the muscles soon passed into firm rigor.'

'Mere alteration in the percentage amount of salt influences the contraction, as the following experiment shows. On April 13 I placed the sartorius in 0·5 per cent. saline solution. For the first three minutes only the faintest contractions occurred. They then for two or three minutes became moderately strong and again grew weak. I then added 25 c.c. 2 per cent. solution of salt to 200 c.c. of 0·5 per cent., which much increased the activity of the contractions. I then returned to 0·5 per cent. solution and in about a minute the contractions became much weaker. I then added 25 c.c. of 2 per cent. solution to 150 c.c. of 0·5 per cent. solution and the contractions became greatly increased. I added another 25 c.c. of 2 per cent. solution and still further increased the contractions. Again I returned to 150 c.c. of 0·5 per cent. solution and the contractions greatly lessened, occurring in paroxysms with quiescent periods of half a minute. I then added 50 c.c. 2 per cent. solution, and again greatly increased the frequency of the contractions. On returning to 150 c.c. of 0·5 per cent. solution contractions ceased but returned for a short time on adding 50 c.c. of 2 per cent. solution of sodium chloride.

On the other hand, lessening the relative amount of NaCl, as by adding distilled water to the saline solution lessens or prevents these muscular contractions. For instance, reducing the 0·6 per cent. to 0·5 per cent. NaCl decidedly lessens the contractions. Here I will record one experiment showing the effect of dilution. I first took a trace with 200 c.c. 0·6 per cent. saline solution, and obtained active contractions. On adding 50 c.c. distilled water, the contractions ceased and failed to return on adding another 50 c.c. and a third 50 c.c. distilled water. On returning to 0·6 per cent. saline, slight contractions recommenced, and the further addition of 2 c.c. of 2 per cent. $BaCl_2$ induced active contractions.

I next tested the effect simply of distilled water on the contractions. I first took a trace with 0·6 per cent. saline solution, and then replaced this by distilled water, when the contractions ceased at once, though in some experiments a few single strong contractions occurred at many minutes' interval. The distilled water however induced some persistent contraction, which passed away on the addition of saline, provided not too long a term had elapsed, before adding the NaCl, i.e., about eight to ten minutes.

When these contractions brought out by immersion in saline cease contractility still persists, and stimulating the muscle with a faradic current the coil, standing at 15 to 20, will excite good contractions. Moreover, the addition of substances like barium chloride at once developes active contraction; indeed barium chloride will produce contractions and persistent spasms much more marked than with saline at the beginning of the experiment.'

'I made twelve experiments to test also the effect of calcium chloride using 1 per cent. solution. I added 2 to 4 c.c. of this to 200 c.c. of saline solution. After testing the effect of simple saline I added at once 4 c.c. of calcium chloride solution. In every instance I tried this solution it almost immediately to a great extent lessened the contractions, and in many instances all but arrested them, in some, quite stopped them. In several cases after a few minutes slight contractions returned but were much less than with saline at the beginning of the experiment. In eight of the experiments at one period after the addition of lime chloride the contraction became literally rhythmic, i.e., the contraction occurred at pretty equal intervals and in much the same force. The following short account of an experiment is a good example of the experiments. On April 5 the sartorius in saline contracted actively. The addition of 4 c.c. of $CaCl_2$ solution at once almost arrested the contractions which were rhythmic in character. I then returned to saline solution, which induced active contractions, and these were at once arrested on the addition of 4 c.c. of calcium chloride solution. Again I placed the muscle in simple saline and again active contractions occurred, which were at once arrested on adding 4 c.c. of 1 per cent. solution of potassium chloride.

The addition of lime chloride arrests not only the rapid contractions but also removes the persistent contraction, so that the trace rises higher above the base line. These effects are shown in fig. 3.

In many experiments made with potassium chloride I find its action is largely influenced by temperature. My experiments have been made generally with the fluid at from 10° to 16° C. Soon after adding 4 c.c. of 1 per cent. solution of KCl to 200 c.c. saline solution the muscle strongly contracts, dragging down the recording lever far below the level reached even when the contractions are well marked with saline; the muscle then slowly relaxes, but before it has much elongated fresh contractions set in, or when at its maximum contraction frequent well marked contractions occur, and these cease and the muscle slowly relaxes, see fig. 4. Then the muscle remains quiescent for a variable

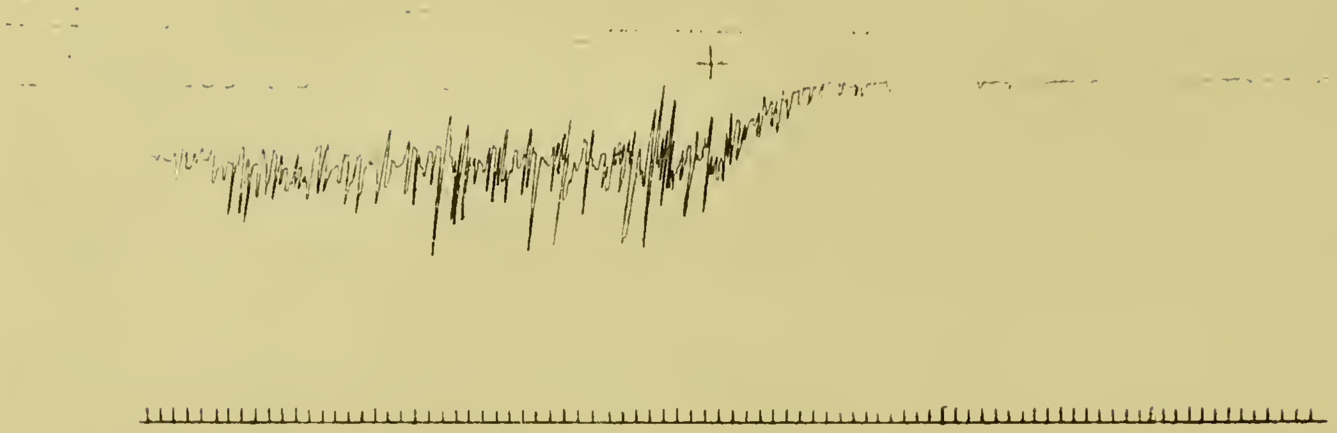

Fig 3

'Shows the effect of saline. At the cross 4 c.c. 1 per cent. $CaCl_2$ were added to 200 c.c. saline.'

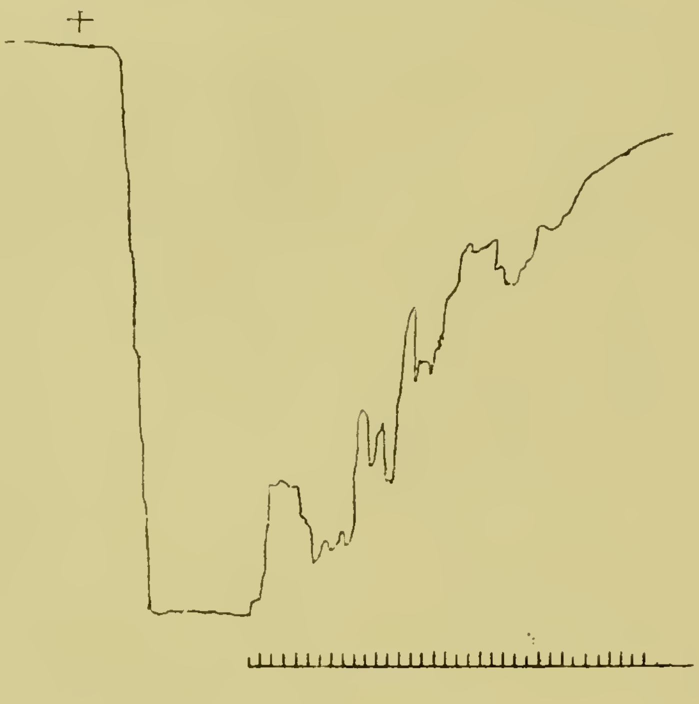

Fig 4

'Effect of adding 4 c.c. 1 per cent. solution of KCl to 200 c.c. saline solution added at the point indicated by a cross.'

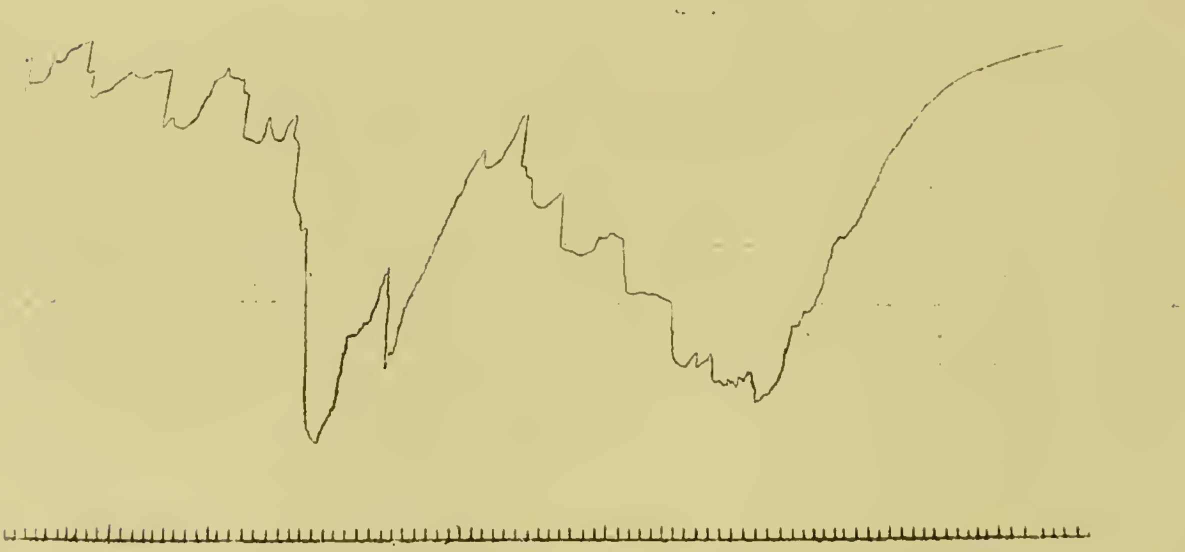

Fig 5

'Later effect of potassium chloride.'

time, ten seconds to one or two minutes, when contractions like those recorded in fig. 5 occur and again the muscle becomes quiet, or after a few minutes again contracts as shown in the figure. By degrees the contractions become less marked and at last merge into a single slight contraction which relaxes slowly. With some muscles only these slighter contractions occur throughout the experiment. With a higher temperature the addition of potassium chloride solution, just like calcium chloride at once arrests the contractions. 1 c.c. of 1 per cent. solution diminishes the contractions greatly, but 2 c.c., i.e., 1 part of KCl in 5000 well-nigh stops them or stops them altogether, and on returning to simple saline solution the contractions recommence actively.

The following experiment is a good example of the influence of potassium chloride. On Feb. 26 a sartorius muscle placed in saline contracted energetically. To the 200 c.c. of saline I then added 2 c.c. of 1 per cent. solution of potassium chloride and the contractions all but ceased. Again I placed the muscle in 200 c.c. of saline solution and active contractions returned. I again added 2 c.c. of potassium chloride solution and again greatly restrained the contractions, yet a third time saline solution restored the contractions, and this final trial stopped the contracting. In the previous experiment the changes were made quickly, but after the addition of 4 c.c. of potassium chloride solution to 200 c.c. saline solution, if the effect is watched for a considerable time, it generally happens that after an interval of two or three minutes contractions return, but occur in groups, a long interval often intervening. The contractions are often very vigorous and rapidly repeated; these outbursts of contractions at long intervals grow weaker and then cease. In some cases the trace is in the form of an irregularly wavy line.

Potassium chloride then whilst modifying considerably the character of the contraction induces very marked contractions, and our previous experiments showed that potassium chloride added to saline solution does not sustain contractility. The difference between calcium chloride and potassium chloride is very striking, as the following experiment shows. On May 6 saline solution excited active contractions, which were at once cut short on adding 4 c.c. of 1 per cent. solution of calcium chloride to 200 c.c. saline. After six minutes I replaced the sartorius in saline and soon after added 4 c.c. of 1 per cent. solution of potassium chloride. This produced the effect shown in fig. 5. I added 4 c.c. $CaCl_2$ solution and at once stopped all contractions. In about four minutes I replaced the muscle in saline without inducing any strong contractions, as generally happens when saline solution replaces saline solution containing potassium chloride 1 part in 5000. Soon after I added 4 c.c. potassium chloride solution and again induced the usual potash effect, which was arrested again though more slowly by adding 4 c.c. of calcium chloride solution. I then again reverted to simple saline without inducing any contractions and again added 4 c.c. potassium chloride solution and induced well-marked potash contraction. These were again stopped though not so rapidly as on the first occasion by adding 4 c.c. of calcium chloride solution. Again I returned to saline, then added potassium chloride solution and again excited well-marked potash contractions, which calcium chloride once more arrested. This action of potassium chloride and the antagonism of calcium chloride I many times verified.'

In the later paper with Buxton it is shown that perfusion of the skeletal muscles with ordinary saline leads to similar contractions to which calcium salt acts again as a sedative. Skeletal muscle was found to differ from cardiac muscle in that, without any addition of calcium salt to the perfusing fluid, the period of relaxation became enormously prolonged, so that the trace became like that obtained from a veratrized muscle. Also, it was found that perfusion with sodium chloride saline alone very rapidly rendered the muscle inexcitable to direct faradization, but that excitability was restored on adding a calcium salt to the perfusing fluid.

Ringer's discoveries on the action of calcium salts upon contractile tissues and living organic processes in general led him to take a great interest in the studies of Hammarsten and J. R. Green on the action of the calcium salts in the coagulation of blood and milk, and to confirm and extend such observations in several important aspects. He showed that this favourable action of calcium salts was a generic property shared in feebler measure by the other members of the calcium group, and that it was to a certain extent antagonised by sodium and potassium, although this antagonism is but a feeble thing compared to that existing in living tissues. He also investigated the action of calcium salts upon metacasein and alkali albumin, showing that, following in exaggerated measure the general rule for inorganic calcium salts, the protein calcium salts are much less soluble at higher temperatures. These papers on the action of calcium upon coagulation contain much interesting material, and many valuable suggestions; they are contained in the *Journal of Physiology,* Vols. XI-XIII.

In the years 1894 and 1895, Ringer, in collaboration first with

Sainsbury,[1] and later with Phear,[2] studied the action upon *Tubifex rivulorum,* and upon growing tadpoles, of various saline constituents, and although the results contained in these papers, in so far as the supposed disintegrative action of distilled water is concerned, were vitiated, as demonstrated soon after by F. S. Locke,[3] by the presence of unsuspected minute traces of copper, yet the experiments on mutual antagonisms contained therein are still valid and valuable, as demonstrated later by Ringer himself (see footnote, p. iii).

In addition to the pioneer work which chiefly forms the subject matter of this sketch of Ringer's scientific labours, he published a text-book of therapeutics which ran through many editions, and from its originality of treatment denoted a scientific aspect of mind towards medicine somewhat rare at that time. His contributions to pathology and medicine are chiefly to be found in the pages of the *Practitioner*, as also more popular expositions of his bio-chemical researches. A biographical notice of Ringer's career, and of his work as a clinician, will be found in the *British Medical Journal*, October 29th, 1910, p. 1384.

BENJAMIN MOORE.

DECEMBER, 1910.

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[1] *Journ. of Physiology*, Vol. XVI, p. 1, 1894.

[2] *Ibid.*, Vol. XVII, p. 423, 1895.

[3] *Ibid.*, Vol. XVIII, p. 319, 1895.

[*From the Journal of Physiology*, Vol. I. No. 1.]

CONCERNING THE EFFECTS ON FROGS OF ARREST OF THE CIRCULATION, AND AN EXPLANATION OF THE ACTION OF POTASH SALTS ON THE ANIMAL BODY.

By SYDNEY RINGER, M.D., *Professor of Therapeutics at University College, and* W. MURRELL, M.R.C.P., *Assistant Physician to the Royal Hospital for Diseases of the Chest.*

PART I.

We have often noticed that different drugs, as potash salts, arsenic, aconitia, produce on frogs the same effects and in the same order. Thus at first slight loss of sensation and of voluntary and reflex action set in; sensation and reflex action greatly diminish and soon become extinct, whilst considerable voluntary power remains; so that an animal without sensation, and therefore also without reflex action, can still hop vigorously. Then voluntary power declines and ceases, the animal becoming completely paralysed. In some experiments made several years ago, the results being intended to serve as standards in other investigations, we found that arrest of the circulation, either by ligature of the bulb of the aorta, or by cutting out the heart, or by bleeding the frog to death by opening the abdominal vein, produced the same effects as the drugs just referred to and in the same order. It occurred to us that these drugs might operate in the same way as mechanical arrest of the circulation, and we at first thought that they might produce their phenomena by arresting the heart's action, since they all paralyse the heart.

We instituted a series of experiments with potash salts to ascertain the truth of this conjecture, and our results are published in the *Journal of Anatomy and Physiology for* 1877. Potash salts we found do not paralyse exclusively by virtue of their power to arrest the heart's action: for after potash poisoning the symptoms come on more quickly and complete paralysis ensues sooner than after mere arrest of circulation by ligature of the aorta. But as potash but slowly arrests the heart this drug should produce paralysis later, and sensation, reflex action, and voluntary power should persist longer than after arrest of the circulation. Moreover, after complete potash paralysis, on examining the web under the microscope, we found the circulation still going on.

How does arrest of the circulation paralyse? It may act in two ways, either by limiting the due supply of oxygen to the tissues or by preventing the removal of the products of retrograde metamorphosis, and these accumulating poison the tissues: in other words, the tissues poison themselves. Both causes will produce the same symptoms in the same order; thus the most highly endowed tissues, the brain and cord, are more dependent on oxidation than other tissues, and are also the first to suffer from the poisonous effect of accumulated waste products.

Do potash salts act by arresting oxidation, or by an affinity for nitrogenous tissues, so destroying their functional activity? If by their affinity for nitrogenous tissues, we should expect the order of the phenomena after potash poisoning would be the same as after mechanical arrest of the circulation; for owing to the common affinity of potash salts for all nitrogenous tissues, those endowed with the highest functional activity must suffer earliest and suffer most, and this too happens with arrest of oxidation by stoppage of the circulation.

We shall venture to attempt to answer these questions, and shall first treat of the effect of arrest of the circulation and endeavour to ascertain by what means it paralyses. Before giving the details of our experiments, it will be convenient to describe in a few words our mode of proceeding. After ligature or excision of the heart, we tested sensation, reflex action, and voluntary power, by pinching a toe or the skin of the leg, and when this failed to excite either voluntary or reflex action, whilst the animal retained considerable voluntary power, we concluded that sensation was abolished.

On mechanical arrest of the Circulation.

In this section we shall first give an account of the order of the symptoms following mechanical arrest of the circulation, and then show that this order varies in different months of the year; then we shall attempt to explain how the arrest of the circulation produces these symptoms.

Our observations were made in June, July, August, September and October of 1876, nearly all between 7 a.m. and 11 a.m. We performed two sets of experiments; one set on unmutilated frogs, the other set on brainless frogs, *i.e.* frogs whose cords we divided opposite the occipito-atlantoid membrane and whose brains we destroyed by passing a wooden peg into the skull, thus serving also to arrest any hæmorrhage.

We must confess that we were not prepared to find how quickly and completely arrest of the circulation paralyses certain parts of the nervous system. The influence of arrest is known to many writers, who indeed refer to it in their papers; but we believe that many experimenters are unaware of its influence and attribute to the direct action of a drug on the nervous system symptoms in part at least due to its effect on the circulation.

We shall first speak of the effect on frogs with the nervous system unmutilated, and then on brainless frogs. We now give a general account of the order of the symptoms, leaving for a subsequent part of our paper a detailed account of the variation that occurred in the colder season. A few minutes after the application of the ligature, both voluntary and reflex action become impaired; the loss of sensation and reflex action rapidly increases in a greater degree than the loss of voluntary power, so that in a short time the loss of sensation and of reflex action becomes complete, whilst the animal still retains considerable voluntary power and can jump vigorously. Sometimes sensation persists when reflex action is destroyed, firm and repeated pinching exciting voluntary but no reflex action. Sensation is lost sooner in the toes than in the calves, and in them than in the thighs, and sooner, we think, in the hind than in the fore extremities. Voluntary power then declines and at last ceases, though for a short time longer, on touching the conjunctiva, the eyelids languidly close.

Whether the loss of reflex action is due to paralysis of the sensory part of the cord, or to paralysis of the portion devoted to reflex action, our experiments on the arrest of the circulation do not enable us to say. As however, after ligature of the abdominal aorta or of the femoral vessels, the nerves of the extremity long retain power to conduct impressions to the cord and brain, and as by means of the ligature they are completely deprived of fresh blood, the loss of sensation after ligature of the heart cannot be due to paralysis of the afferent nerves, but must be due to paralysis of the sensory part of the cord or to the sensory centres in the brain. If due to paralysis of the sensory centres in the brain, the loss of reflex action must be due to paralysis of reflex centres of the cord.

We now give a summary of our observations in the following table:

Effect of Ligature of Aorta on Frogs with the Nervous System entire.

Date. 1876.	Sensation and reflex action diminished.	Sensation and reflex action destroyed.	Voluntary power diminished.	Voluntary power destroyed.
June 21	9 min.	?	9 min.	35 min.
June 22	5 min.	?	5 min.	15 min.
June 23	3 min.	?	7 min.	27 min.
Average	5·7 min.		7 min.	25·7 min. or 31
July 12	6 min.	11 min.	6 min.	25 min.
July 12	11 min.	16 min.	11 min.	31 min.
Average	8·5 min.	13·5 min.	8·5 min.	28 min.
Aug. 2	11 min.	13 min.	11 min.	22 min.
Aug. 2	4 min.	5 min.	4 min.	31 min.
Aug. 2	4 min.	14 min.	4 min.	30 min.
Aug. 16	5 min.	7 min.	4 min.	17 min.
Average	6 min.	9·8 min.	5·7 min.	25 min.
Sep. 9	2 min.	7 min.	4 min.	43 min.
Sep. 9	2 min.	7 min.	3 min.	52 min.
Sep. 9	3 min.	5 min.	3 min.	42 min.
Sep. 12	2 min.	6 min.	6 min.	49 min.
Sep. 12	2 min.	3 min.	4 min.	47 min.
Sep. 14	3 min.	3 min.	4 min.	44 min.
Average	2·3 min.	5·2 min.	4 min.	46 min.
Oct. 10			2 min.	36 min.
Oct. 21			4 min.	45 min.
Oct. 21			3 min.	20 min.
Oct. 22			4 min.	65 min.
Oct. 22			3 min.	48 min.
Oct. 23			3 min.	42 min.
Average			3·1 min.	42·6 min.

From this table we extract the following averages:

Month.	Sensation and reflex action destroyed.	Voluntary power destroyed.
July	13 min.	28 min.
Aug.	9 min.	27 min.
Sep.	5 min.	46 min.

This table shows very clearly how much sooner sensation and reflex action is lost than voluntary power. Thus to take the month of September, we find on an average of six observations that sensation and reflex action were lost in five minutes, whilst voluntary power perished in forty-six minutes.

We were surprised however to find in our October experiments that sensation lasted as long as voluntary power; thus pinching would always excite voluntary movement as long, or almost as long, as any remained. We are unable in these October experiments to decide how long reflex action continued; for it is difficult to ascertain whether in a voluntary act excited by stimulation there is likewise any reflex movement. The phenomena of arrest of circulation therefore vary somewhat in October from preceding months. Thus during June, July, August, and September reflex action and sensation ceased early, whilst much voluntary power persisted; but in October sensation lasted much longer, nearly as long as any voluntary power. Though sensation persisted longer in October than in the previous months, yet the phenomena occurred in the same order. Sensation first disappeared at the toes, and whilst pinching these parts excited neither voluntary nor reflex action, pinching the ankles produced voluntary movement; next sensation left the ankles whilst it still continued higher up, and then gradually disappeared, leaving the lower part of the leg before that nearer the trunk. When sensation was destroyed in the feet and ankles considerable voluntary power often remained.

How is this paralysis produced? Conceivably in two ways—by cerebral "shock," or by arrest of oxidation.

Is it due to cerebral shock? We believe not, for the following reasons. The operation of tying the aorta, with the consequent distension of the heart, would cause, it might be thought, sufficient shock to produce widespread paralysis.

To test whether the paralysis is due to cerebral "shock" we varied our experiments. Instead of tying the bulb of the aorta, we either excised the heart or made a wound into it, destroying its action. The results of our experiments we give in the following table.

Frogs allowed to bleed to death by Excision or a Wound of the Heart.

Date.	Sensation and reflex action began to diminish.	Sensation and reflex action destroyed.	Voluntary power diminished.	Voluntary power destroyed.	
June 15	10 min.	55 min.			Cord divided and frog pegged.
June 15		35 min.			,, ,,
Aug. 2	6 min.	11 min.	6 min.	38 min.	Nervous system entire.
Aug. 14	2 min.	18 min.			Cord divided and frog pegged.
Sep. 9		3 min.	5 min.	52 min.	Nervous system entire.
Sep. 11	35 min.	52 min.			Cord divided and frog pegged.
Sep. 12		4 min.	6 min.	54 min.	Nervous system entire.

It thus appears that excision, or a wound of the heart, produces the same symptoms, in the same order, and in much the same time, as ligature of the aorta. It may be said, however, that excision or a wound of the heart might cause shock sufficient to produce general and complete paralysis. We therefore performed another experiment; we opened the abdominal vein and allowed the frog to bleed to death, an operation which could produce very little if any "shock," and yet on looking at the accompanying table it is seen that paralysis occurred, the symptoms following the same order and occurring in much about the same time as with ligature of the aorta.

Date.	Reflex action destroyed.	Voluntary power impaired.	Voluntary power destroyed.	Sensation destroyed.
Sep. 13	1½ min.	1½ min.	44 min.	3½ min.

Arrest of circulation whilst it does not paralyse by "shock," yet considerably depresses the cord through its influence on the brain, as the following experiments show.

We first divided the cord opposite the occipito-atlantal membrane, and in some cases passed a peg of wood into the cavity of the skull, destroying the brain and checking hæmorrhage. Then we watched the animal till perfect reflex action returned, and this if permanently weakened by the operation led to the rejection of the animal. After the re-establishment of reflex action, we tied the bulb of the aorta; and the following table shows our results.

Cord divided opposite the Occipito-Atlantal Membrane, before Ligature of the Heart.

Date.	Reflex action began to decline.	Reflex action destroyed.	
June 15		45 min.	Also pegged.
June 15		60 min.	,, ,,
August 9	4 min.	21 min.	,, ,,
August 11	4 min.	21 min.	,, ,,
August 14	3 min.	15 min.	,, ,,
August 14		14 min.	Not pegged.
August 16	2 min.	12 min.	,, ,,
August 16	2 min.	13 min.	,, ,,
Average	3 min.	16 min.	
Sep. 9	10 min.	56 min.	,, ,,
Sep. 11	30 min.?	54 min.	,, ,,
Sep. 11	17 min.	48 min.	,, ,,
Sep. 12	5 min.	66 min.	Pegged.
Sep. 14	15 min.	70 min.	Pegged.
Average	15 min.	59 min.	

From this table it appears that the arrest of circulation in the brain exercises a strong depressing influence on the cord. Thus in August, in frogs whose nervous system was entire, reflex action was destroyed in 10 minutes, whilst it lasted 16 minutes in brainless frogs. This effect in September was still more manifest, for whilst in entire frogs reflex action was lost on an average in 5 min., in brainless frogs it persisted on an average 59 min. What is the character of the influence? Does arrest of circulation depress the reflex action of the cord by stimulation of Setschenow's inhibitory centre? To test this we made the following experiments.

After ligature of the bulb of the aorta, when weakness of reflex action had set in, we divided the cord opposite the occipito-atlantal membrane, and instead of restoring reflex action the operation completely abolished it.

The direct paralysing effect of arrest of circulation on the cord is about as rapid as its effects on the brain, as the following table shows.

Month.	Complete paralysis in frogs with nervous system entire.	Complete loss of reflex action in brainless frogs.
July.	28 minutes.	
August.	27 minutes.	16 minutes.
September.	46 minutes.	59 minutes.

How does arrest of the circulation paralyse? The circulation has a twofold office: to convey nutritive matter and oxygen to the tissues, and to remove from them the products of retrograde metamorphosis. Does arrest of the circulation paralyse by arresting oxidation, or by allowing the excrementitious substances to accumulate, so as to destroy function; to allow indeed the tissues to poison themselves?

The arrest of oxidation is sufficient to explain the paralysis, for no functional activity is possible without oxygen. Now in arrest of the circulation no fresh oxygen is conveyed to the tissues; hence when the oxygen is consumed in the tissues and in the stationary blood no further function is possible.

It may be urged perhaps that the well-known "salt frog" or "bloodless frog" experiment disproves this conclusion. For the blood may be completely washed out from the vessels of a frog and be replaced by a one per cent. solution of common salt, and yet the animal may live several days. But Oertmann* has shown that the solution of common salt absorbs sufficient oxygen for the wants of the animal which can just maintain life for at least two or three days.

Does the accumulation of the products of retrograde metamorphosis in the tissues assist in producing the paralysis? Our experiments do not enable us to answer the question.

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* *Pflüger's Archiv*, xv. p. 381.

PART II.*

On the Action of Chloride of Potassium on Frogs.

We shall now speak of chloride of potassium; but as all potash salts act in the same way, the following remarks are applicable to them all.

We shall show how closely the effects of chloride of potassium correspond to those of mechanical arrest of the circulation. Both produce the same effects, which occur in the same order; in both, the order of the symptoms in the winter months varies from their order in the summer months.

In the *Journal of Anatomy and Physiology* for 1877, we showed that during the summer months, chloride of potassium, like mechanical arrest of the circulation, at first weakens both voluntary power, reflex power, and sensation; the loss of sensation and reflex action rapidly goes on till they are exhausted, whilst considerable voluntary power still remains, so that an animal without sensation and insusceptible of reflex action, can still hop vigorously. In this communication we shall not give the details of any experiments showing the effect of chloride of potassium during the summer months, but shall insert a table from our previous communication in the *Journal of Anatomy and Physiology.*

Average of twelve experiments with chloride of potassium administered by the stomach.

Reflex action and sensation destroyed.	Voluntary power impaired.	Voluntary power destroyed.
4·8 minutes.	2·7 minutes.	33 minutes.

This table clearly shows that during the summer months voluntary power continues for nearly thirty minutes after complete loss of sensation, in this respect corresponding to the effects of arrest of the circulation during the summer months.

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* Owing to Dr Murrell's engagements he was unable to assist me in these investigations after the early part of November. S. R.

We now give the details of experiments showing that the order of the symptoms in October is the same after chloride of potassium as after arrest of the circulation; that in fact sensation, instead of being early lost, persists as long, or almost as long, as voluntary power.

Before giving the details of these experiments, we first point out a few particulars touching our mode of proceeding. We employed a 1 in 5 solution of chloride of potassium administered hypodermically. The animals after the poisoning were kept moist in the warm laboratory, and were covered with a glass shade. The following table gives the results of the October and November experiments.

Date. 1877.	Sensation and reflex action diminished.	Sensation and reflex action destroyed.	Voluntary power diminished.	Voluntary power destroyed.	Weight of frog in grammes.	Dose in grains.	Proportionate dose.
Oct. 16	4 min.	23 min.	4 min.	37 min.	17	$\frac{2}{5}$	$\frac{1}{656}$
Oct. 16		53 min.	7 min.	60 min.	20	$\frac{2}{5}$	$\frac{1}{772}$
Oct. 17*		19 min.	12 min.	33 min.	11	$\frac{1}{5}$	$\frac{1}{849}$
Oct. 17		14 min.	5 min.	24 min.	14	$\frac{2}{5}$	$\frac{1}{540}$
Oct. 18		6 min.	10 min.	38 min.	$30\frac{1}{2}$	$\frac{3}{5}$	$\frac{1}{784}$
Oct. 18		24 min.	4 min.	36 min.	28	$\frac{3}{5}$	$\frac{1}{720}$
Oct. 23		6 min.	8 min.	23 min.	16	$1\frac{2}{5}$	$\frac{1}{176}$
Oct. 23	11 min.	21 min.	11 min.	26 min.	25	$1\frac{1}{5}$	$\frac{1}{321}$
Nov. 1	3 min.	43 min.	3 min.	53 min.	34	$1\frac{2}{5}$	$\frac{1}{374}$
Nov. 2	1 min.	34 min.	1 min.	34 min.	25	$1\frac{2}{5}$	$\frac{1}{275}$
Nov. 28		56 min.		66 min.	20	$\frac{2}{5}$	$\frac{1}{772}$
Nov. 28		56 min.		76 min.	32	$\frac{3}{5}$	$\frac{1}{823}$
Nov. 28		27 min.		31 min.	23	$\frac{4}{5}$	$\frac{1}{443}$
Average		29 min.		41 min.			

This table shows that after the use of the same salt, sensation and reflex action continued much longer than in the summer months; that in fact sensation and reflex action often continued almost, and in some cases quite, as long as voluntary power. The averages of the thirteen experiments give the duration of sensation 29 minutes, of voluntary power 41 minutes.

Now, as we have seen, the same phenomena occur in mechanical arrest of the circulation, for during October sensation lasts as long, or almost as long, as voluntary power, whilst, in the summer months, sensation is soon destroyed. This close similarity in the action of chloride of potassium and mechanical arrest of the circulation, naturally suggested the idea that they might both operate in the same way.

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* Potash solution injected under the skin of the abdomen instead of under that of the back.

Potash salts are powerful arresters of the heart's action. Is their paralysing action due to their stopping the heart's action? If not due to their action on the heart, do they, like mechanical arrest of the circulation, act by preventing oxidation?

We hope to prove that potash salts do not paralyse simply by arresting the heart's action, but that, having an affinity for all nitrogenous tissue, they destroy its functions, and then, as in mechanical arrest of the circulation, the most highly organized structures suffer most and earliest, so that the paralytic symptoms will occur in the same order after mechanical arrest of the circulation as after poisoning by a potash salt.

Of course it is not here maintained that the arrest of the heart's action by chloride of potassium does not play any part in the production of the paralytic symptoms. As the paralysis comes on sooner after potash than after ligature of the heart, and as, moreover, the paralysis from potash is complete whilst the circulation visible by the microscope goes on in the web of the frog's foot, we only maintain that the action of chloride of potassium on the heart is insufficient to account for the paralysis.

We shall now endeavour to show that potash paralyses the central nervous system, the nerves and the muscles, and that this paralysis is not due to the action of the potash salts arresting the heart's action. We shall treat first of the paralysis of the central nervous system.

Guttmann, in his careful investigations, has shown that potash salts paralyse the central nervous system. Thus, after complete paralysis from potash salts, the motor nerves will still conduct impressions to the muscles, and the muscles will still contract on the direct application of galvanism, showing that the paralysis is due. not to the action of the potash salt on the motor nerves or muscles, but on the cord.

Our investigations fully confirm this conclusion. Guttmann also finds that after ligature of the abdominal aorta or of the femoral vessels, before the administration of the potash salt, the posterior protected limbs become completely paralysed, although neither their nerves nor muscles had been poisoned by the potash salt, having been protected from the poison by the ligature of the blood-vessels. This experiment shows that potash salts paralyse the cord, and that the loss of reflex action is not due to paralysis of the afferent nerves, for these are protected in the hind limbs by the ligature of the blood-vessels, yet irritation of the skin of the posterior limbs fails to excite reflex action.

The general paralysis, then, after poisoning by chloride of potassium, is due to paralysis of the central nervous system. Now this paralysis is not due to arrest of the heart by the potash salt; for, as we have elsewhere shown, the paralysis from chloride of potassium becomes complete much sooner than after arrest of the circulation by ligature of the heart; moreover, when the potash paralysis is complete, the circulation is still visible in the web of the frog's foot (*Journal of Anatomy and Physiology*, 1877). Chloride of potassium, therefore, has a direct paralysing action on the central nervous system.

We shall next treat of the action of chloride of potassium on the muscles, to show that, as in the case of the nervous centres, it paralyses the muscles directly, and not indirectly by stopping the heart; for muscular irritability ceases far sooner after poisoning by chloride of potassium than after arrest of the circulation by ligature or excision of the heart.

In the *Journal of Anatomy and Physiology* for 1877, we showed that the topical application of a solution of chloride of potassium immediately suspends sensation in the skin of the immersed leg. Thus, pinching any part of a leg after thirty seconds immersion, or a little longer, excites neither voluntary nor reflex action; soon the immersed leg grows feeble, and quickly becomes completely paralysed, whilst the general power of the animal is very little affected. In many instances, if chloride of potassium has not been too freely absorbed, the animal completely recovers, proving that the potash has simply suspended, not destroyed function. If when the immersed leg has become completely paralysed, the muscles are laid bare, it is found that they do not contract, or scarcely contract, on the application of a strong faradaic current, evidencing the absorption of chloride of potassium, and that its topical action has suspended the contractility of the muscle. The poisonous effect of chloride of potassium on muscle is also shown when a solution (1 in 5) is injected under the skin, when the muscles with which it comes in contact become at once completely paralysed, and after death, on removing the skin, the muscles will not contract on the direct application of a strong faradaic current. The solution easily diffuses itself through the loose connective tissue, and thus the paralysing effects spread widely.

Now this topical effect cannot result from the action of the potash on the heart, for during the partial paralysis it beats well; moreover, it need scarcely be added, were the paralysis due to arrest of the heart, the paralysis should have been general. Thus chloride of potassium appears to be a muscle-poison.

We then performed numerous other experiments to strengthen our conclusion that chloride of potassium is a muscle-poison.

We performed two sets of experiments, one on frogs with the nervous system intact, the other on brainless frogs. We tied a ligature very tightly round the thigh of the left leg close to the trunk, to prevent the poison penetrating to that limb through either the circulation or by means of diffusion into the tissues, and then poisoned the animal by injecting the solution (1 in 5) into the left axilla. We then noted when complete paralysis ensued, and how long muscular irritability continued in both lower legs. The results are given in the following table:

Chloride of Potassium Solution 1 *in* 5.

Table showing the Duration of Muscular Irritability in Poisoned and Non-Poisoned Legs of Frogs. Solution injected into Left Axilla.

Date 1877.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Paralysis complete in	Muscular irritability in poisoned leg continued.	Muscular irritability in ligatured non-poisoned leg continued.
Nov. 19	$\frac{2}{5}$	25	$\frac{1}{965}$	45 min.	7 hours	9 hours
Nov. 19	$\frac{3}{5}$	42	$\frac{1}{1080}$	49 min.	7 hours	9 hours
			On Brainless Frogs.			
Nov. 20	$\frac{1}{5}$	22	$\frac{1}{1698}$	25 min.	5 hours	7 hours
Nov. 21	$\frac{2}{5}$	44	$\frac{1}{1689}$	55 min.	16 hours	50 hours
Nov. 21	$\frac{1}{5}$	19	$\frac{1}{1466}$	70 min.	25 hours	48 hours
Nov. 22	$\frac{4}{5}$	37	$\frac{1}{714}$	47 min.	26 hours	38 hours
Nov. 23	$\frac{3}{5}$	40	$\frac{1}{1029}$	68 min.	58 hours	76 hours
Nov. 23	$\frac{2}{5}$	26	$\frac{1}{1003}$	58 min.	14 hours	74 hours
			Average	54·5 min.	24 hours	49 hours

If the ligature is not applied tight enough to cut quite through the muscles, it does not appear to afford complete protection against the salt, which either poisons the ligatured limb by its passage through the blood-vessels with the blood or by diffusion through the tissues. For the most part, though not always, we tightened the ligature so as to cut nearly down to the bone; and the occasional omission will, we believe, to some extent explain the rather variable results. We extract the averages from this Table:

Average duration of muscular irritability in the poisoned unligatured leg of brainless frogs	24 hours.
Average duration of muscular irritability in the non-poisoned ligatured leg	49 hours.

This table shows that muscular irritability persists many hours longer, indeed twice as long, in unpoisoned than in poisoned muscles.

We then varied our experiments. We ligatured the left thigh of a brainless frog in two places close to the trunk, amputated the leg between the ligatures, and then injected the poison into the left axilla. The results are given in the following Table; and it will at once be evident how closely these experiments agree with the figures in the previous Table.

Chloride of Potassium Solution 1 *in* 5.

Table showing the comparative Duration of Muscular Irritability in the Unpoisoned Amputated Leg and the Poisoned Unamputated Leg in the Frog.

Date 1877.	Dose in grains.	Weight of Frog in grammes.	Proportionate dose.	Paralysis complete.	Muscular irritability in poisoned leg continued	Muscular irritability in amputated leg continued
Nov. 21	$\frac{2}{5}$	18*	$\frac{1}{694}$	42 min.	9 hours	44 hours
Nov. 24	$\frac{2}{5}$	25	$\frac{1}{965}$	65 min.	14 hours	50 hours
Nov. 24	$\frac{3}{5}$	23	$\frac{1}{592}$	63 min.	38 hours	24 hours
Nov. 24	$\frac{4}{5}$	32	$\frac{1}{617}$	45 min.	38 hours	38 hours
Nov. 24	$\frac{4}{5}$	40	$\frac{1}{772}$	53 min.	14 hours	50 hours
Nov. 24	$\frac{4}{5}$	27	$\frac{1}{521}$	50 min.	38 hours	48 hours
Nov. 24	$\frac{3}{6}$	24	$\frac{1}{617}$	45 min.	40 hours	52 hours
			Average	52 min.	25·5 hours	47 hours excluding No. 3.

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* Weight without the non-poisoned amputated leg.

Thus the average muscular irritability lasted in the poisoned limb .... 25·5 hours.
In the amputated unpoisoned leg .... 47 hours.

Thus observations on the duration of muscular irritability of the frog's calf, even when the injection was made in the axilla, and therefore at a distance from the tested part, prove the poisonous action of chloride of potassium on muscle.

In the previous experiments we tested the electric irritability in the gastrocnemius and other muscles of the lower leg. But in one experiment we found that after poisoning with chloride of potassium, the muscular irritability declined much sooner in the thigh than in the lower leg, and far sooner in the thigh of the frog when the injection was administered under the skin of the back, than when injected in the axilla. We always observed that the muscles close by the place of injection became first affected, those at the greatest distance being last paralysed. This suggests strongly the idea that the poison spreads by imbibition and diffusion through the tissues and not through the circulation. Being a powerful heart-poison, the heart is soon weakened or arrested, so that but little potash is distributed through the body by the circulation. For were the poison carried by the circulation, then all parts would become simultaneously paralysed, whilst if it spread by imbibition, the parts adjacent to the injection would be the first to suffer, while distant parts would become affected last, the very order of the phenomena observed after poisoning by this salt. It is evident, therefore, that the muscles of the lower leg lying some distance from the axilla are not the best adapted to test the influence of potash salts. Moreover, the scanty tissues around the knee are ill adapted to allow the penetration of the potash, the connective tissue being dense, binds the skin close to the bone, impeding the diffusion of the chloride to the muscles of the lower leg.

We next performed a different series of comparative experiments. We injected the solution into the left axilla of frogs, and then ascertained when muscular irritability ceased in the anterior limbs, in the thighs, and in the lower legs. At the same time we made a comparative test-experiment to ascertain how long muscular irritability endures in the muscles of unpoisoned frogs, whose circulation had been arrested by extirpation of the heart.

The results are given in the following table :

Table showing the Duration of Muscular Irritability and the order of its disappearance, in Frogs poisoned with Chloride of Potassium, and in Frogs with Extirpated Heart.

Frogs poisoned with Chloride of Potassium.

Date 1877.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Paralysis complete in	Muscular* irritability persisted in anterior limbs	Muscular irritability persisted in thighs	Muscular irritability persisted in calf	Sensation continued
Nov. 29	$\frac{2}{5}$	20	$\frac{1}{772}$	66 min.	3 hours	6 hours	6 hours	
Nov. 29	$\frac{3}{5}$	32	$\frac{1}{823}$	76 min.	3 hours	3 hours	3 hours	
Nov. 29	$\frac{4}{5}$	23	$\frac{1}{444}$	31 min.	3 hours	3 hours	4 hours	
Nov. 30	$\frac{4}{5}$	21	$\frac{1}{405}$	58 min.	1 hour	10 hours	14 hours	
Nov. 30	$\frac{4}{5}$	33	$\frac{1}{636}$	77 min.	lost immediately	9 hours	38 hours	
Nov. 30	$\frac{4}{5}$	22	$\frac{1}{424}$	74 min.	3 hours	6 hours	14 hours	
Nov. 30	$\frac{3}{5}$	31	$\frac{1}{797}$	58 min.	lost immediately	5 hours	10 hours	
			Average	63 min.	1·8 hours	6 hours	8·5 (excluding No. 5)	
				Frog with extirpated heart.				
Nov. 29				53 min.	16 hours	16 hours	16 hours	48 min.
				Pithed and Pegged Frogs with extirpated heart.				
Nov. 30				100 min.	16 hours	38 hours	42 hours	
Nov. 30				102 min.	16 hours	32 hours	40 hours	
			Average	85 min.	16 hours	28·6 hours	32·6 hours	

* After complete paralysis.

The muscular irritability, it may be observed, declined with unusual rapidity in the experiments of Nov. 29. The four frogs in question were taken from the same batch, had been kept in the same conditions, and after the experiment were all laid side by side and treated in all respects in the same way; hence we look upon this series of experiments as trustworthy. The room became very warm, which probably helped to destroy muscular irritability. In the experiments of other dates we took care to keep the window open and the room cool.

These experiments, like the foregoing, show that after poisoning with chloride of potassium, the muscular irritability ceases sooner than in frogs with arrest of circulation from excision of the heart.

Thus with the potash the muscular irritability ceased on an average

in the thigh in 6 hours,
in the calf in 8·5 hours;

whilst in non-poisoned frogs, with extirpated heart, the muscular irritability ceased

in the thigh in 28·6 hours,
in the calf in 32·6 hours.

We now give the general results of the foregoing series of experiments.

Muscular irritability ceased in a poisoned unamputated leg in	25·5 hours.
In the amputated unpoisoned leg in	47 „
Muscular irritability ceased in a poisoned lower leg in	24 „
In ligatured unpoisoned leg in	49 „

These tables clearly show that chloride of potassium does not paralyse the muscles simply by stopping the heart's action, but that it is a direct poison to the muscles.

Not only is chloride of potassium a muscle-poison, but it is also a direct poison to the motor nerves, and does not simply affect these nerves indirectly by arresting the circulation, as we shall now proceed to show.

In the previous experiments with chloride of potassium we noticed that the sciatic nerve ceased to conduct impressions, whilst the calf-muscles still responded strongly on the direct application of galvanism; whilst in the case of another unpoisoned frog which was pithed and pegged and its heart cut out at the time whilst the other frog was being poisoned, the sciatic still conducted freely. But we thought it better to make a few special comparative experiments to settle this point, and the results are shown in the following Table.

Two frogs were pithed and pegged and their hearts excised. At the same time two other frogs were poisoned by injecting the chloride of potassium solution under the skin of the back. We then tested three times daily the sciatic nerve of each frog, first through the skin, and when the nerve failed to conduct, we laid it bare, and again tested it by direct application of the electrodes.

Table showing the Effect of Chloride of Potassium on the Motor Nerves.

Frogs poisoned with Chloride of Potassium.

Date 1877.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Paralysis complete.	Sciatic nerve ceased to conduct on direct stimulation.	Muscular irritability ceased in anterior limb.	Muscular irritability ceased in thighs.	Muscular irritability ceased in calf.
Dec. 1	$\frac{4}{5}$	48	$\frac{1}{926}$	57 min.	4 hours	4 hours	7 hours	7 hours
Dec. 1	$\frac{3}{5}$	21	$\frac{1}{540}$		3 hours	3 hours	3 hours	4 hours

Frogs Pithed and Pegged and the heart extirpated.

Date 1877.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Paralysis complete.	Sciatic nerve ceased to conduct on direct stimulation.	Muscular irritability ceased in anterior limb.	Muscular irritability ceased in thighs.	Muscular irritability ceased in calf.
Dec. 1				100 min.	38 hours	52 hours	64 hours	86 hours
Dec. 1				74 min.	52 hours	52 hours	64 hours	78 hours

These experiments clearly show that chloride of potassium is a powerful paralyser of motor nerve-fibres; for whilst the sciatic nerve of a frog poisoned with this salt ceased to conduct, even under strong galvanic stimulation, in 4 and 3 hours respectively, the sciatic nerve of a frog pithed and pegged, with its circulation arrested by extirpation of the heart, conducted for 38 and 52 hours respectively.

This Table also confirms our conclusion that this salt is a muscle-poison, for in the poisoned frogs muscular irritability ceased in the thighs seven and three hours respectively, whilst in the unpoisoned frogs in each instance it persisted 64 hours.

Chloride of potassium is also a paralyser of sensory nerves.

We have elsewhere shown that the chloride paralyses also the afferent nerves, for its topical application suspends or abolishes sensation, and if it can act thus, *à fortiori* it can operate when carried to the nerve by the blood, but being in a more dilute and weaker state, its effects will be less obvious.

How do potash salts paralyse the heart? In the same manner as they paralyse the other structures, by an affinity for all the nitrogenous tissues. The more highly endowed nervous ganglia are the first to suffer, hence small doses will arrest the heart whilst the muscular tissue will still respond to galvanic stimulation; but if into the jugular vein a large quantity of potash salt is at once introduced, it will destroy not only the functions of the nervous structures, but also muscular contractility. Thus Guttmann found that the injection of small doses into the jugular vein of warm-blooded animals paralyses through the nervous system; whilst Traube found that the injection of a large dose paralyses also the muscular tissue so that it failed to contract on the application of galvanism. These experiments strongly support the views we have advanced.

If it seems strange that chloride of potassium produces such profound effects on frogs whilst it appears so harmless a drug for man, we need merely point out that our frogs were given a quantity proportionate to from 3 to 9 oz. for a man weighing 150 lbs., and so large a dose thrown at once into the circulation would doubtless profoundly affect the chemical condition of the blood and the functional activity of the organs.

We have shown that chloride of potassium paralyses the nervous centres, the nerves, and the muscles; the cord and brain suffering first, then the nerves, and lastly the muscles. This paralysis is conceivably producible in three ways, (1) By arresting the circulation, (2) by checking oxidation, (3) by the direct action of the potash on the tissues. Now though potash is a powerful heart-poison, still it does not act entirely by stopping the heart, for the paralysis ensues more rapidly after poisoning with potash than after arrest of the circulation, either by ligature of the aorta or by extirpation of the heart.

Does the potash act by preventing oxidation, or by its direct action on the tissues? If by arresting oxidation, it is easy to understand that potash will then paralyse sooner than mechanical arrest of the circulation; for some oxygen remains in the blood, on which the tissues can draw for a time, whilst after poisoning by chloride of potassium this appropriation of the oxygen may be at once prevented. These experiments do not enable us to decide this question, though we think it probable that this salt acts on the nitrogenous tissues, altering their chemical nature, and so destroying or arresting their function.

This salt then has a marked affinity for all nitrogenous tissues; does it manifest an especial action on some? We conclude that it has an equal affinity for all nitrogenous tissues, and for this reason: in mechanical arrest of the circulation we find the tissues become paralysed in the same order as after poisoning by chloride of potassium. After arrest of the circulation the tissues become paralysed in the order of their vital endowments; those most highly endowed succumbing first, and those least endowed last. Now if potash has an equal action on all nitrogenous tissues, we should expect it to act in this very order. The most highly endowed tissues would suffer first and most readily, the less highly endowed the least and last. Now this is the actual order in which potash affects the different structures, and therefore we venture to conclude that potash is a simple poison to all nitrogenous tissues, having the same affinity for all.

The order of the onset of the symptoms after potash poisoning depends of course on the order in which individual parts of the nervous system are affected. Sensation and reflex action, as we have seen, are in the summer months much earlier affected than voluntary power. Are we to conclude, therefore, that potash paralyses the sensory and the reflex before the voluntary centres?

We have pointed out that these symptoms in summer come on in the same order from mechanical arrest of the circulation, and that the tissues afterwards suffer in proportion to their physiological endowments. We have seen also that potash affects all nitrogenous tissues, those most highly endowed being the first to suffer. We might perhaps, therefore, be inclined to conclude that the reflex and sensory centres are more highly endowed than the voluntary centres, and that this difference might account for the order of the symptoms both after potash poisoning and mechanical arrest of the circulation.

In the section treating of mechanical arrest of the circulation, we showed that the early depression of the reflex function which occurs in the summer months is due to the effect of arrest of circulation on the brain; thus, on tying the aorta after division of the cord, reflex action continues much longer than in a frog with undivided spinal cord. We concluded that mechanical arrest of the circulation, through its influence on the brain, induces early loss of reflex action, and that we cannot assume that the reflex portion of the cord is more highly endowed than the brain, and therefore perishes sooner than the brain from mechanical arrest of the circulation. We suggest that, in the summer months, the early extinction of reflex action by chloride of potassium is not due to the direct paralysing effect of this salt on the reflex centres before the brain, but depends on the action of the salt on the heart arresting the circulation.

We now give a summary of the conclusions arrived at in this section on the action of Chloride of Potassium:

1. It paralyses all nitrogenous tissues.

2. It acts by an equal affinity for all protoplasm; and it destroys the tissues in the order of their vital endowments.

3. It arrests the heart, owing to its common action on all protoplasm, not from a special action on that organ.

4. Arrest of the circulation, itself a paralysing influence, must in some degree assist the direct action of the potash on the tissues.

5. By arresting the circulation, potash, probably at an early stage, depresses the reflex function of the cord, in the summer months, indirectly through arrest of the circulation in the brain.

PART III.

Experiments to show how Chloride of Potassium spreads through the tissues.

BY SIDNEY RINGER.

In the previous part of this paper reasons have already been adduced suggesting the idea that this salt (and other potash salts) spreads by imbibition or diffusion and not through the agency of the circulation. The potash probably quickly arrests the heart and prevents the passage of the potash through the blood-vessels; for were the potash conveyed through the circulation, all parts of the animal should be equally and simultaneously affected. Now the parts nearest the seat of injection become first affected and the most distant parts last. I therefore per-

Table showing the Diffusion of Chloride of Potassium through the Tissues. Frog pithed and pegged and Heart cut out before poisoning with solution of Chloride of Potassium 1 in 5.

Date.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Paralysis complete.	Paralysis complete in anterior limb.		Paralysis complete in posterior limb.				
							Thigh.		Lower leg.		
					Right.	Left.	Right.	Left.	Right.	Left.	
Dec. 3	$\frac{4}{5}$	30	$\frac{1}{579}$	60 min.	2 hours	42 hours	8 hours	40 hours	12 hours	62 hours	Solution injected into right axilla.
Dec. 3	$\frac{4}{5}$	24	$\frac{1}{463}$		10 hours	7 hours	16 hours	16 hours	16 hours	11 hours	Solution injected into left axilla.
Dec. 4	$\frac{3}{5}$	23	$\frac{1}{591}$		16 hours	16 hours	16 hours	16 hours	50 hours	50 hours	Solution injected under skin of back.
				Average	9 hours	21 hours	13 hours	24 hours	26 hours	41 hours	

formed the following experiments to elucidate this question. I pithed (divided the cord opposite the occipito-atlantoid membrane) and pegged some frogs and extirpated their hearts, thus completely arresting the circulation, and then injected some of the potash solution, in two instances into the axilla, and in a third under the skin of the back, and then tested all the limbs to ascertain how soon the muscular irritability ceased. At the same time I performed three comparative test experiments, pithing, pegging, and extirpating the heart, but without poisoning by potash. These frogs I also tested to ascertain if the muscles of the unpoisoned animal retained their irritability longer than the poisoned frogs.

Table showing Comparative Duration of Muscular Irritability in the Frog pithed and pegged, and with the Heart cut out.

Date.	Paralysis complete.	Paralysis complete in both anterior limbs.	Paralysis complete in both thighs.	Paralysis complete in both lower legs.
Dec. 3	95 min.		76 hours	88 hours
Dec. 3	90 min.	34 hours	50 hours	76 hours
Dec. 4	63 min.	57 hours	60 hours	60 hours
	Average	45 hours	62 hours	74 hours

The foregoing table shows that in frogs poisoned with chloride of potassium the muscles lose their irritability much sooner than unpoisoned animals, a fact well exemplified in these figures, extracted from the preceding table:

Muscular irritability ceased in the …………	Anterior extremities.		Thighs.		Lower legs.	
	Poisoned frog.	Unpoisoned frog.	Poisoned.	Unpoisoned.	Poisoned.	Unpoisoned.
	21 hours	45 hours	24 hours	62 hours	41 hours	74 hours

These figures themselves are sufficient to show that chloride of potassium can diffuse itself and paralyse the muscles without passing through the circulation, for in these experiments the heart being extirpated and the circulation therefore completely abolished, yet the muscles become much sooner paralysed than the muscles of frogs subjected to mechanical arrest of the circulation without poisoning.

The order in which the paralysis occurred in these poisoned frogs proves the same thing. Thus in the first experiment the injection was made into the right axilla, and the right anterior extremity became paralysed in 2 hours and the left in 42, and whilst the right thigh became paralysed in 8 hours the left required 40. In the right calf too paralysis set in in 12 hours, whilst the left required 62. That is to say, the tissues on the side of the injection became paralysed much sooner than the tissues on the opposite side of the body. This is what we should have expected, since in these frogs from which the heart had been previously removed, the solution must spread by diffusion. Now these frogs were pithed—that is, the cord was divided opposite the occipito-atlantoid membrane—and the front of the chest was opened to reach the heart. These operations had so divided the skin and the subjacent connective tissue, that but little was left for the potash to soak through, hence the anterior limb opposite the seat of injection took many hours before it became paralysed.

It must, I think, be admitted that this salt and probably other salts can freely travel by imbibition and so produce their effects at a distance. Mere ligature of the vessels is clearly not sufficient to protect a limb from the poison. Hence all the experiments conducted in this way are untrustworthy, including those of Guttmann referred to in this paper. But whilst Guttmann's experiments performed in this manner are fallacious, still his conclusions are correct, for other reasons which are detailed in this paper.

We have, I think, in the foregoing experiments an explanation of the slow paralysis of muscle induced by potash. It may be fairly said, if potash is poisonous to muscle it should at once manifest its effects, and speedily reach its culminating point. The explanation of its slow action is, I believe, not difficult. Being a powerful poison both of nerve and muscle tissue, when absorption takes place and the potash salt reaches the heart, this organ becomes paralysed, and thus the passage of the salt through the circulation is prevented. It then spreads slowly by diffusion through the tissues, and thus by degrees very tardily poisons them. The effects of other nerve- and muscle-poisons are doubtless thus modified, and the rate of paralysis will depend not only on their poisonous action, but likewise on their diffusibility and the distance the nerves and muscles are situated from the point of subcutaneous injection.

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THE ACTION OF ARSENIATE OF SODA AND ARSENIOUS ACID ON FROGS.

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IN the *Archiv für Anat. Physiolog.*, &c. 1866, p. 481, Sklarek records some experiments concerning the action of arsenic on frogs. He says, that in about five minutes after injecting it, the animal lies flat with extended extremities, and without breathing. Pinching or other irritation excites neither reflex action nor voluntary motion, though much voluntary power remains, for on lifting the animal, or withdrawing a leg, or turning the frog on its back, it displays active voluntary movements. In a short time, however, the animal becomes completely paralysed. Arsenic, therefore, paralyses first sensation and reflex action, and sometime afterwards voluntary power.

Now paralysis takes place in this order after mechanical arrest of the circulation by ligature, or by excision of the heart. Arsenic being a powerful paralyser of the heart, we were induced to undertake this series of investigations to ascertain whether arsenic paralyses simply by arresting the heart's action. Our experiments show that arsenic does not paralyse simply by its action on the heart, but that, like potash, it has a poisonous action on all nitrogenous tissues, and destroys their function.

We commenced our experiments in October, 1877. We first experimented with arseniate of soda, using a solution of the same strength as that employed by Sklarek, but we obtained very different results from those described by him. We then tried arsenious acid dissolved by the aid of a small quantity of soda, and with this form of the drug we obtained effects similar to those recorded by Sklarek. In this paper we shall describe, first the action of arseniate of soda, and then of arsenic, and point out in what respects they differ; then we shall adduce experiments proving that arsenious acid is a protoplasmic poison, destructive of the function of nitrogenous tissue.

The action of Arseniate of Soda on Frogs.

We employed the soda salt, since soda, unless used in very large quantities, does not paralyse frogs, whilst even a small quantity of a potash salt produces complete paralysis. We injected under the skin the quantity used by Sklarek in his experiments, but we obtained very different results. Thus paralysis set in slowly, and sensation persisted as long, or almost as long, as voluntary power. Sensation first ceased in the toe and foot, whilst in the calf and thigh it still remained good. Then in some cases sensation ceased before the loss of voluntary power, but in others continued as long as voluntary power. Table I. shows the weight of the animal, and the dose of arsenic administered.

Table I. Solution injected hypodermically under skin of back.

Arseniate of Soda, 1 in 20 solution.

Date.	Weight of animal in grammes.	Dose in grains.	
Oct. 23	28½	$\frac{1}{5}$	
„ 23	21	$\frac{1}{10}$	
„ 23	33	$\frac{3}{5}$	
„ 24	32	$\frac{3}{20}$	
„ 24	27	$\frac{3}{20}$	1 in 40 solution.

Though we administered large doses, from $\frac{1}{10}$th to $\frac{3}{5}$ths of a grain, still for several hours the poison produced no effect, the frogs in these experiments remaining unaffected 4½, 11, 4½, 7, and 7 hours respectively, and then paralysis set in.

In the first experiment, after 11 hours, only very slight reflex action remained.

In the second, after 13 hours, only slight reflex action remained, and next morning the animal was found dead.

In the third, the animal died in 11 hours.

In the fourth, voluntary movement was extinguished in 11 hours, but reflex action remained good. This frog was found dead next morning.

In the fifth, after 11 hours, slight voluntary power remained, and reflex action was unimpaired. Next morning we found the frog dead.

These experiments show that for several hours (4 to 11), after poisoning with arseniate of soda, the animal remains unaffected; that complete paralysis is not established in less than 13 hours; and that voluntary power disappears before reflex action.

The action of Arsenious Acid on Frogs.

The effects of arseniate of soda being so different from those described by Sklarek, we next experimented with arsenious acid. As arsenious acid is so little soluble in water, we were obliged to add an alkali, and chose soda, avoiding potash, this base having such a powerful action on all the tissues. Mr Gerrard prepared for us a solution containing a grain of arsenious acid to five of water, adding hydrate of soda in the proportion of 1 in 22.

This solution we injected under the skin of the back, and we give the results in Table II.

The experiments there tabulated show that in poisoning by arsenious acid, sensation and reflex action continue longer than voluntary action; for pinching the leg excited reflex action after complete loss of voluntary power, indicating that the afferent nerves still conducted impressions. In this table we have used the term "sensation" to point out that the afferent nerves are still active.

The effects therefore of arsenious acid dissolved by the aid of soda are very different from the effects of arseniate of soda. With arseniate of soda, the paralysis does not begin till four or more hours, and does not become complete for twelve or more hours, whilst after arsenious acid paralysis becomes complete in 40 minutes.

It might be just possible, we thought, that the powerful effect of the arsenious acid solution was due to the rather large quantity of soda it contained. To ascertain whether the soda played any part in the paralysis, we experimented with a solution of soda prepared for us by Mr Gerrard, containing the same proportion of soda as in the arsenious acid solution. This we injected under the skin of the back of two frogs, employing the same proportionate dose of soda to the weight of the animal, as in the previous experiments with arsenious acid. Beyond the primary shock, lasting about a minute, this injection produced no effect; therefore the paralysis following the injection of the arsenious acid solution is entirely due to the arsenic. The arsenious acid solution produced a far greater shock than the simple soda solution, and it occurred to us that this was due to the great concentration,

Table II.

Arsenious acid dissolved with aid of liquor sodæ. Solution containing 1 *grain of arsenious acid in* 5 *of the solution. Hydrate of soda* 1 *in* 22.

Date.	Dose in grains.	Weight of animal in grammes.	Proportionate dose.	Loss of sensation and reflex action begun.	Loss of sensation and reflex action complete.	Loss of voluntary power begun.	Loss of voluntary power complete.
Oct. 25	$\frac{1}{5}$	24	$\frac{1}{1852}$	6 min.	55 min.	6 min.	45 min.
„ 25	$\frac{2}{5}$	23	$\frac{1}{887}$	5 min.	33 min.	1 min.	28 min.
„ 27	$\frac{1}{10}$	23	$\frac{1}{3551}$	7 min.	56 min.	7 min.	56 min. } 1 in 10 solution
„ 27	$\frac{1}{5}$	15	$\frac{1}{1158}$	7 min.	52 min.	7 min.	47 min. } 1 in 10 solution
„ 30	$\frac{1}{20}$	10	$\frac{1}{3088}$	5 min.	23 min.	5 min.	23 min. } 1 in 20 solution
„ 30	$\frac{1}{10}$	15	$\frac{1}{2316}$	5 min.	29 min.	5 min.	29 min. } 1 in 20 solution
Nov. 5	1	21	$\frac{1}{324}$		36 min.	4 min.	41 min. 1 in 5 solution
			Average	5·8 min.	40·5 min.	5 min.	38 min.

Experiments with solution of hydrate of soda 1 *in* 22.

Oct. 26	$\frac{1}{22}$	21	No effect except the primary frightened state lasting one minute.
	$\frac{1}{11}$	21	No effect except the primary frightened state lasting one minute.

therefore in our two last experiments we diluted the solution to 1 in 10 with the effect of greatly reducing the shock.

But whilst the effects of arseniate of soda differ greatly from those of arsenious acid dissolved in soda, the difference consists in the rapidity of the occurrence of the effects, for in both cases the afferent nerves retained their conducting power as long, or even longer, than voluntary power continued.

In this respect, therefore, our observations differ from those of Dr Sklarek, who found that sensation and reflex action were abolished in about six minutes. The reconcilement of this difference is, we think, to be found in the time of year the experiments were made. We presume (for unfortunately Dr Sklarek does not give the season) that his investigations took place in the months of May, June, July, or August, whilst ours were made in October. We have found similar differences, as we have already said, in the effect of arrest of circulation, and in potash poisoning.

We had hitherto used very strong solutions of arsenious acid, 1 in 5, 1 in 10, and 1 in 20. The 1 in 5 solution appeared to have a strong topical action; and to test if this were so, we injected some of the solution under the skin of the thigh. After complete paralysis we stripped off the skin, and examined the muscles. Those the solution had reached were yellowish and quite hard, and did not in the slightest degree contract on the application of a strong galvanic current. We therefore used a much weaker solution, namely, 2 per cent., the strength employed by Sklarek. We should have used this weaker form at first; but, with so weak a solution, we could not give our frogs anything like the doses he administered.

Mr Gerrard made us a 2 per cent. solution with just sufficient liquor sodæ to dissolve the arsenic. We injected five minims of this under the back of a frog, and after the induction of complete paralysis, we examined the muscles, and they appeared unaffected; moreover, the back and thigh muscles contracted under galvanism, as well as the muscles of the rest of the body. This solution therefore has no immediate topical action. We therefore made a series of observations with this 2 per cent. solution, always injecting it under the skin of the back, close to the posterior lymph-hearts.

We first give a short account of the symptoms induced by these small doses. Soon after the injection the animal becomes quiet, but sensation, reflex action, and voluntary power, are unimpaired. In ten to twenty minutes the animal often gapes, and keeps its mouth open, and sometimes puts its paws into its mouth. It looks as if sick, and two frogs actually vomited. Then, after twenty to thirty minutes, voluntary power declines, sensation continuing apparently quite unimpaired till all movement ceases, or nearly ceases. It is difficult to tell when reflex action begins to decline, as it is often difficult to decide between a voluntary and a reflex act. A few hours after death a very strong post mortem rigidity sets in.

Table III.

Solution of arsenious acid 2 per cent. dissolved with the aid of smallest quantity possible of liquor sodæ.

Date.	Dose in grains.	Weight of animal in grammes.	Proportionate dose.	Loss of sensation begun.	Loss of sensation and reflex action complete.	Loss of voluntary power begun.	Loss of voluntary power complete.
Nov. 6	$\frac{1}{10}$	31	$\frac{1}{4786}$	4 min.	42 min.	4 min.	42 min.
,, 8	$\frac{1}{25}$	18	$\frac{1}{6948}$	32 min.	52 min.	26 min.	52 min.
,, 8	$\frac{1}{50}$	20	$\frac{1}{15440}$	38 min.	57 min.	38 min.	57 min.
,, 9	$\frac{1}{50}$	34	$\frac{1}{26248}$	70 min.?	130 min.	33 min.	130 min.
,, 9	$\frac{1}{50}$	40	$\frac{1}{30880}$	77 min.?	108 min.	28 min.	108 min.
,, 9	$\frac{1}{50}$	30	$\frac{1}{23160}$	48 min.	58 min.	26 min.	58 min.
,, 9	$\frac{1}{50}$	29	$\frac{1}{22388}$	75 min.	80 min.	30 min.	80 min.
,, 10	$\frac{1}{12}$	33	$\frac{1}{6114}$		49 min.	4 min.	49 min.
,, 10	$\frac{1}{25}$	21	$\frac{1}{8106}$		41 min.	6 min.	41 min.
,, 13	$\frac{1}{25}$	31	$\frac{1}{11966}$		60 min.	20 min.	60 min.
,, 14	$\frac{1}{8}$	43	$\frac{1}{5311}$		34 min.	5 min.	34 min.
,, 15	$\frac{1}{25}$	26	$\frac{1}{10036}$		42 min.	2 min.	42 min.
				Average	62·7 min.		62·7 min.

Table IV. shows that even small doses varying from $\frac{1}{4000}$ to $\frac{1}{30000}$ of the weight of the animal quickly paralyse, for on the average of these twelve experiments paralysis was complete in 62 minutes. We were astonished to find how very fatal arsenious acid is to frogs, for $\frac{1}{30000}$ of the weight of the animal produced complete paralysis in 108 min., and $\frac{1}{80000}$ killed the frog on the third day, and we were likewise astonished to find how far more poisonous arsenious acid is than arseniate of soda, $\frac{1}{5}$ gr. of this salt requiring 11 hours to produce almost complete paralysis.

Table IV.

Solution of arsenious acid $\frac{1}{2}$ per cent. dissolved with the smallest quantity possible of liquor sodæ.

Date.	Dose in grains.	Weight of animal in grammes.	Propor-tionate dose.	
Nov. 10	$\frac{1}{100}$	33	$\frac{1}{50900}$	Died on second day
„ 10	$\frac{1}{100}$	$21\frac{1}{2}$	$\frac{1}{33100}$	Died on second day
„ 10	$\frac{1}{200}$	26	$\frac{1}{80288}$	Died on third day
„ 12	$\frac{1}{200}$	27	$\frac{1}{83376}$	Died on third day
„ 12	$\frac{1}{200}$	25	$\frac{1}{77200}$	

This general paralysis is due to the action of the poison on the central nervous system, for after complete paralysis, on the direct application of galvanism, the muscles still contract and the motor nerves still conduct.

We are now in a position to attempt to answer in part the question we put at the beginning of this paper—Does arsenic paralyse simply by arresting the heart?

We must first compare the times respectively when paralysis became complete after poisoning by arsenic, and by mechanical arrest of the circulation. In Table II. we find that after poisoning by arsenic, paralysis became complete on an average in 40 minutes, and in a subsequent set of experiments, where a smaller dose of arsenic was administered, the results of which are given in Table III., paralysis became complete on an average in 62 minutes. These experiments were made in October and the first half of November. In the month of October mechanical arrest of the circulation, as we have shown in a previous paper, produces complete paralysis in the average time of 42 minutes. With regard to these figures, it is evident that arsenic may indirectly paralyse the central nervous system by its paralysing action on the heart, for mechanical arrest will produce complete paralysis as soon, and sometimes sooner, than after poisoning by arsenic.

We next performed the following experiments to attempt to decide this question.

We poisoned three frogs by injecting a solution of arsenic under the skin of the back. Two became completely paralysed respectively in 56 and 60 minutes, and the third was greatly paralysed, though some movement continued, in 60 minutes. At the times just stated we placed the web under the microscope and found the circulation completely arrested in all three frogs, and on opening the chest the heart was found motionless and widely distended with blood. These experiments likewise tend to show that the general paralysis may be due to arrest of the heart.

In some other experiments, however, after complete paralysis we found the heart beating well, in one instance at 30 per minute. Unfortunately we omitted to examine the circulation in the web.

As after the general paralysis the motor nerves conducted impressions, and caused the muscles to contract vigorously, it is obvious that the general paralysis is due to the condition of the central nervous system, and not to the condition of the nerves and muscles; moreover, as in some instances after general paralysis, the heart still beat fairly well, we conclude that arsenic does not act through the heart, but is a direct paralyser of the central nervous system.

How are we to explain the varying effects of arsenic on the heart? We have seen that in some cases the heart is speedily arrested, whilst in others it continues to beat after complete paralysis. This difference is, we think, due to the dose. A large dose is quickly absorbed and conveyed in large quantity to the heart, arrests it at once, leaving very little to be distributed through the circulation, the general paralysis being then mainly due to this arrest of the circulation. In other cases, under a smaller dose, the heart is not so quickly stopped, but enough arsenic is distributed by the circulation to paralyse the central nervous system before the heart.

We shall next show that arsenic is both a muscle and a nerve poison, as well as a direct paralyser of the central nervous system.

In some experiments on frogs with a 2 per cent. and a still weaker solution of arsenious acid, dissolved with the aid of a small quantity of liquor sodæ, we noticed some curious effects. Even so early as six or eight hours after death strong *rigor mortis* set in, and this suggested the idea that arsenic is a muscle as well as a nerve poison, and to test the validity of this conjecture we performed the following experiments.

We poisoned two frogs with $\frac{1}{12}$ and $\frac{1}{25}$ gr. of arsenious acid, in a 2 per cent. solution, injected under the skin of the back, close to the posterior lymph-hearts. A summary of the effects is given in Table III. Nov. 10. As soon as the two animals became completely paralysed, we divided the cord of another frog, opposite the occipito-atlantal membrane, and passed the blade of the forceps into the skull, destroying the brain. The wound bled freely, and we took no steps to check the hæmorrhage. This frog we used as a standard to compare the muscular contractility of the arsenicized frogs.

Seven minutes after complete paralysis we tested the muscles through the skin, and found that the muscles of the brainless frog contracted rather better than those of the arsenicized frogs. In an hour and an half the muscles of the brainless frog contracted decidedly better than the muscles of the arsenicized frogs. In three hours and a half the muscles of the brainless frog still contracted energetically with a weak current; whilst the strongest current excited only very feeble and scarcely visible contractions in the arsenicized frogs. We noticed also in the arsenicized frogs that the weak muscular contraction was excited equally with a comparatively gentle current as by the strongest the battery could compass. Next morning, eighteen hours after complete paralysis, we found the arsenicized frogs quite stiff; whilst very good, though relatively weaker reflex action continued in the brainless frog. The strongest current failed to excite any muscular contraction in the muscles of the arsenicized frogs, whilst a weak current produced strong muscular contraction on direct application to the muscles in the brainless frog. We then stripped off all the skin from the arsenicized frogs, and during this process we noticed the curious fact, which we shall hereafter refer to, that the cuticle came off very easily, indeed it could be rubbed off. Then we again tested the muscles of the anterior and posterior extremities, and failed to produce the slightest contraction with the strongest current from a one-celled du Bois-Reymond induction coil.

This experiment seemed to render it highly probable that arsenious acid is a muscle-poison, though it must be admitted the comparative experiment with the brainless frog was hardly a satisfactory test to determine whether arsenic acts directly on the muscles or by arresting the heart; for though we allowed the animal to bleed freely from the wound when we divided the cord, we did not cut out the heart, and so though but little blood was left in the vessels, probably this little circulated and supplied the tissues with oxygen. We therefore performed some more experiments free from this possible fallacy.

Each morning we poisoned a frog by injecting the 2 per cent. solution into the connective tissue of the axilla, to avoid the direct topical action on the hind legs. When this animal became completely paralysed we cut the cord of another frog opposite the occipito-atlantal membrane, destroyed the brain, and then extirpated the heart. The two frogs were then laid side by side, and during the day we frequently tested them with the interrupted current, first through the skin, and when this failed to contract the muscles, we removed the skin, and applied the electrodes directly to the muscles. We also tested the nerves.

We give the results of our experiments in Table V. :

Table V. Duration of muscular contractility after poisoning with arsenious acid, 2 per cent. solution dissolved with a small quantity of liquor sodæ.

Date.	Weight of frog.	Dose of arsenic in grains.	Propor-tionate dose.	Complete paralysis.	Muscular contractility ceased in
Nov. 10	33	$\frac{1}{12}$	$\frac{1}{6114}$	49 min.	$3\frac{1}{2}$ hours*
„ 10	21	$\frac{1}{25}$	$\frac{1}{8106}$	40 min.	$3\frac{1}{2}$ hours*
„ 13	31	$\frac{1}{25}$	$\frac{1}{11966}$	60 min.	$9\frac{1}{2}$ hours
„ 14	43	$\frac{1}{8}$	$\frac{1}{5311}$	34 min.	$5\frac{1}{2}$ hours
„ 15	26	$\frac{1}{25}$	$\frac{1}{10036}$	42 min.	$5\frac{3}{4}$ hours
			Average	45 min.	5·6 hours
Duration of muscular contractility in brainless frogs with extirpated heart.					
Nov. 12					25 hours
„ 13					48 hours
„ 14					29 hours
„ 15					15 hours
				Average	29 hours

This Table (V.) shows that in frogs poisoned with arsenious acid, muscular irritability ceases in from three to nine hours, whilst the irritability persists in brainless frogs with extirpated heart, from one to two days, or an average time of 29 hours. How does arsenic arrest muscular contraction? Does it act by arresting the heart's action? The Table shows that arsenic does not paralyse the muscles by arresting the heart: for if arsenic paralyses the muscles only through its action on the heart, the muscular irritability should persist as long in the arsenicized frogs as in the frogs with extirpated heart. The loss of muscular irritability, therefore, is not due to arrest of the circulation, but to the direct action of the arsenic on the muscles; in other words, arsenic is a muscle-poison.

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* Had not quite ceased, but the strongest current of a one-celled du Bois-Reymond battery excited only the slightest contraction.

We next devised another set of experiments further to test the validity of our conclusion, that arsenious acid is a muscle-poison. We divided the cord of a frog opposite the occipito-atlantal membrane, and passed a wooden peg into the cavity of the skull, destroying the brain and preventing hæmorrhage. Now we tied a ligature tightly round the left thigh, close to its junction with the trunk, to arrest the circulation, then we injected the arsenious acid into the left axilla, and, from time to time, tested the muscular irritability of the ligatured and the non-ligatured poisoned legs, and in Table VI. we give the results.

Table VI. Table showing the duration of muscular irritability in a poisoned and unpoisoned leg of pithed and pegged frogs.

Date.	Dose of arsenious acid in grains.	Weight of frog in grammes.	Propor-tionate dose.	Reflex action destroyed.	Muscular irritability in poisoned leg ceased in	Muscular irritability in ligatured non-poisoned leg ceased in
Nov. 15	$\frac{1}{12}$	24	$\frac{1}{4446}$	27 min.	17 hours*	43 hours
„ 15	$\frac{1}{25}$	25	$\frac{1}{9650}$	44 min.	17 hours*	41 hours
„ 16	$\frac{1}{25}$	27	$\frac{1}{10422}$	50 min.	7 hours	16 hours
„ 16	$\frac{1}{50}$	21	$\frac{1}{17833}$	53 min.		
„ 17	$\frac{1}{12}$	37	$\frac{1}{6855}$	38 min.	9 hours	40 hours
„ 17	$\frac{1}{12}$	35	$\frac{1}{6484}$	35 min.	16 hours	31 hours
			Average	41 min.	13 hours	34 hours

Here we see that the muscles of the poisoned posterior limb lost their irritability in an average time of 13 hours, whilst in the limb tightly ligatured the muscles retained their contractility an average time of 34 hours. The results shown in this Table clearly demonstrate that arsenic is a muscle-poison.

It is worthy of notice, that after complete destruction of reflex action, the muscular irritability continued equally manifest in both hind legs for three to six hours.

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* An interval of eleven hours, namely from 8.30 p.m. to 7.30 a.m., elapsed between our observations; at 8.30 p.m. the muscles contracted well, next morning at 7.30 a.m. contraction had ceased; thus these figures are not very correct, and probably muscular irritability lasted a shorter time than here stated.

Our experiments, moreover, show that arsenious acid is also a nerve poison. In several previous experiments we noticed that the motor nerves in arsenicized frogs became paralysed many hours before the motor nerves of frogs with mechanical arrest of the circulation. We determined, however, to make some further experiments intended especially to ascertain the action of arsenious acid on motor nerves. We poisoned frogs, by injecting a 2 per cent. solution under the back, and simultaneously prepared some test-frogs, by dividing the cord opposite the occipito-atlantal membrane, and then cut out the heart to arrest circulation. Then we tested the sciatic nerve with the interrupted current through the skin, morning, noon, and night.

It may be said perhaps that we should not have tested the conductivity of the sciatic nerve through the skin, that we should have exposed it and isolated it on a piece of glass. Now this would have invalidated our experiment, for by isolating the nerve we should have separated it from the tissues and cut off the blood-supply, thus preventing the poison reaching the nerve either through the circulation or by imbibition. Arsenic, like potash, quickly paralyses the heart, so that but little of the poison reaches the tissues through the blood. Its paralysing effects are due to its diffusion by imbibition through the tissues, isolation therefore would have prevented the play of the arsenic on the nerve. Moreover, as we treated the test-frogs in the same way, our experiments are so far trustworthy, as a reference will show that on applying the electrodes over the sciatic of arsenicized frogs the nerve ceased to conduct impressions to the lower leg and foot muscles sooner than in the case of the unpoisoned test frogs.

Table VII. exhibits our results.

Although we poisoned and tested six frogs, it will be seen that only four are available in evidence. In two experiments, between the interval of our visits, the muscular irritability, on the direct application of the electrodes, had ceased, and it is obvious we could test the conductivity of the sciatic nerve so long only as the muscles could contract. In the four remaining experiments we found that the sciatic nerve ceased to conduct several hours before the muscles lost their contractility. We infer, therefore, that in these instances the sciatics were paralysed; moreover, on comparing the time it took to paralyse the sciatics of arsenicized frogs with the time it took to paralyse the sciatics of test-frogs, we found that the sciatic nerves of arsenicized frogs ceased to conduct impressions many hours before the same nerves in the test-frogs. Thus in the arsenicized frogs, the sciatics became paralysed, respectively, in 4, 4, 14, and 10 hours, giving an average of

Table VII. Action of Arsenious Acid on Motor Nerves and Muscles.

Date.	Dose in grains.	Weight of animal in grammes.	Sciatic nerve ceased to conduct.	Muscular irritability ceased in				Place of injection.
				Anterior extremities.	Muscles of back.	Thigh.	Lower leg muscles.	
Feb. 26	$\frac{1}{12}$	28	Left 4 hours Right 8 hours	4 hours 4 hours		8 hours 8 hours	8 hours 8 hours	Right axilla
,, 26	$\frac{1}{12}$	32	Left 4 hours Right 4 hours	4 hours 4 hours		5 hours 5 hours	8 hours 8 hours	Left axilla
,, 26	$\frac{1}{25}$	28	4 hours	4 hours		4 hours	4 hours	Under skin of back
,, 26	$\frac{1}{12}$	38	8 hours	8 hours		8 hours	8 hours	Under back
,, 27	$\frac{1}{50}$	31	Left 14 hours	10 hours		10 hours	R 10 hours L 19 hours	Under back
,, 27	$\frac{1}{50}$	30	10 hours	10 hours		10 hours	16 hours	Under back
			Average	6 hours		7 hours	10 hours	
Test Frogs. Cord divided and heart excised.								
Feb. 26			34 hours	38 hours	28 hours	34 hours	54 hours	
,, 26			28 hours	24 hours	28 hours	24 hours	40 hours	
		Average	31 hours	31 hours	28 hours	29 hours	47 hours	

8 hours, whilst the sciatics of the test-frogs with arrested circulation conducted for 34 and 28 hours respectively, giving an average of 31 hours. We conclude, therefore, that arsenic is a paralyser of motor nerves.

These experiments on nerve incidentally confirm our conclusion that arsenic is a muscle poison, as the following averages extracted from Table VII. shows.

Table VIII (Resumé of Table). The average duration of muscular contractility.

	Anterior limb.	Thigh.	Lower leg.
Arsenicized frogs	6 hours	7 hours	10 hours
Unpoisoned frogs	31 hours	29 hours	47 hours

On the strength of the following experiment we suggest that arsenious acid is also a poison to afferent nerves.

We immersed the left leg of a frog, for two minutes, in a two per cent. solution of arsenic, and on removing it we found sensation abolished, for pinching it neither excited voluntary nor reflex movement, whilst the animal still retained complete voluntary power over the limb. The animal continued in this condition an hour and a half. On examining it in five hours and a half, we found it completely paralysed.

The muscles became paralysed in the following order: left leg, right thigh and back in nine hours, and right lower leg muscles in 17 hours. The right sciatic became paralysed in 12 hours. It is evident that the poison did not spread by means of the circulation but passed by imbibition up the left immersed leg, poisoning next the muscles of the back and right thigh with the right sciatic nerve, and lastly some hours afterwards reaching and poisoning the right lower leg muscles. This experiment also shows that arsenious acid is a nerve poison, for the right sciatic early ceased to conduct impressions to the lower leg muscles.

As in this experiment the local application of an arsenious acid solution destroyed sensation in the immersed leg, it is, we suggest, at least feasible that when conducted to the sensory nerves, either by the circulation or by imbibition, the poison would equally destroy sensation.

The preceding experiments, we venture to think, clearly establish that arsenious acid is a protoplasmic poison, affecting first the more highly organized nervous centres, next the nerves, and last the muscles. The evidence tending to show that arsenious acid directly paralyses the central nervous system, being not so conclusive as we could wish, we were led, through a suggestion of Dr Burdon Sanderson, to perform another set of experiments. Arsenious acid, as we have said, quickly arrests the heart, so that but little arsenious acid finds its way through the circulation. We therefore injected a solution into the vessels. We first divided the cord and passed a peg through the wound into the cavity of the skull; we then opened the chest and introduced a cannula into the bulb of the aorta, and having securely tied it, we injected the solution, containing one per cent. of common salt, and a half per cent. of arsenious acid dissolved by the aid of a very small quantity of liquor sodæ.

At the same time, we performed some test-experiments to ascertain the effect of the injection of a one per cent. solution of common salt. These frogs were in all other respects treated like the arsenicized frogs. The results are shewn in Table IX.

Table IX. Solution of Arsenious Acid injected through the blood vessels.

Date.	Reflex action ceased.	Sciatic nerve paralysed.	Muscular irritability ceased in			Strength of solution.
			Anterior limbs.	Thigh.	Lower leg muscles.	
Mar. 6	8 min.		5 hours	5 hours	5 hours	$\frac{1}{2}$ p. c.
„ 7	immediately injection finished.		5 hours	5 hours	5 hours	„
„ 7	„	5 hours	5 hours	5 hours	10 hours	„
„ 9	„		5 hours	5 hours	5 hours	$\frac{1}{5}$ p. c.
Test frogs injected with solution of common salt.						
Mar. 4	48 min.	25 hours	40 hours	40 hours	42 hours	$\frac{3}{4}$ p. c.
„ 7	60 min.	28 hours	28 hours	36 hours	40 hours	1 p. c.
Average	54 min.	26·5 hours	34 hours	38 hours	41 hours	

Now these experiments confirm our previous conclusions. They show that arsenious acid directly poisons the central nervous system, and does not affect it only by arresting the heart. Thus in the frogs, injected with common salt, whose circulation was of course arrested when we divided the bulb to insert the cannula, reflex action persisted for 48 and 60 minutes respectively, whilst in the case of the frogs whose vessels were injected with the arsenious acid solution, the reflex action in three cases was destroyed when we had finished the injection, and in the remaining case it lasted only eight minutes.

These, like some of the previous experiments, show that arsenious acid is a muscle-poison, for muscular irritability was lost in the arsenicized frogs in five hours, whilst it persisted in the frogs injected with common salt solution, in the anterior limbs 34 hours, in the thighs 38 hours, and in the lower leg muscles 41 hours.

Whilst our experiments show that arsenious acid is a poison to all nitrogenous tissues, they fail to prove whether it is equally poisonous to all tissues, or whether it manifests an especial action on some, as on the brain, cord, &c., or even on parts of these. Our experiments were not designed, and do not help us, to decide these points.

How does arsenious acid destroy the vitality of nitrogenous tissues? Does it kill by preventing oxidation, or by combining with the tissues? Our observations do not enable us to answer this question. One fact we noticed seems to suggest that it destroys in neither way. Thus conductivity of the motor nerves and the muscular contractility do not cease for some hours after poisoning by arsenious acid. In our early experiments, in which we administered the poison hypodermically, we thought that by arresting the heart quickly the poison did not get diffused by means of the circulation, but by imbibition through the tissues. This slow diffusion of the poison seems to explain the tardy action on the nerves and muscles; but this solution is not strictly correct, for even when the poison was injected through the vessels, yet paralysis did not for several hours set in in the nerves and muscles, though, indeed, sooner than in frogs poisoned hypodermically. We may conclude, therefore, that even when brought into immediate contact with the tissues, arsenic, unless in a very concentrated state, requires some hours to produce paralysis. Now, if arsenic acted solely by arresting oxidation, or by combining with the tissues, we should expect it to produce its effects much earlier.

We may draw attention to a curious effect of arsenious acid on the skin of frogs. We have said that in our experiments we noticed that a few hours after the hypodermic injection we could strip off the cuticle with the greatest readiness over every part of the body. We give in the following Table the results of our observations. We tied a tight ligature round one hind leg of some of the frogs to protect the tissues below from the effect of the poison.

Table X. Action of Arsenious Acid on the Cuticle.

Date.	Proportional dose.	Begun.		Begun.
Nov. 13	$\frac{1}{11966}$			9 hours
„ 14	$\frac{1}{5311}$			$5\frac{1}{2}$ hours
„ 15	$\frac{1}{10036}$			42 min.
		Begun in non-ligatured leg.	Begun in ligatured leg.	Begun on trunk.
„ 15	$\frac{1}{4446}$	12 hours	None in 24 hours	$3\frac{1}{2}$ hours
„ 15	$\frac{1}{9650}$	12 hours	25 hours	$3\frac{1}{2}$ hours
„ 16	$\frac{1}{10422}$	$5\frac{1}{2}$ hours	None	$5\frac{1}{2}$ hours
„ 16	$\frac{1}{6853}$	9 hours	None	9 hours
„ 17	$\frac{1}{6484}$	5 hours	None	$5\frac{1}{2}$ hours
	Average	8·7 hours		5·4 hours

Table X. shows that desquamation begins on the trunk on an average in five and a half hours, in the leg on an average in eight and a half hours. That the desquamation is due to the arsenious acid is well shown by the absence of desquamation in the limb surrounded by a tight ligature. Moreover, that the desquamation depends on the arsenious acid is well shown also by the experiments recorded in Table XI. In these experiments we killed the frogs by dividing the cord and cutting out the heart, and then watched carefully for several days without detecting any desquamation.

Is this desquamation a peculiar result of arsenious acid, or does it follow the hypodermic injection of other poisons? We tested chloride of potassium, aconitia, extract of duboisia, and acetate of strychnia.

Table XI. Desquamation in dead but unpoisoned frogs.

Date.	None in
Nov. 30	72 hours
„ 30	72 hours
Dec. 1	58 hours
„ 1	58 hours
„ 3	48 hours
„ 3	48 hours
„ 8	84 hours
„ 8	84 hours

Table XII. shows the effect of chloride of potassium.

Table XII. Desquamation.

Chloride of Potassium 1 *in* 5.

Date.	Cuticle strips off at place of injection.	No general desquamation took place during	Desquamation in
Nov. 20		24 hours	
„ 21		53 hours	
„ 21		53 hours	
„ 21		48 hours	
„ 22		30 hours	48 hours
„ 23		24 hours	
„ 23	72 hours	72 hours	
„ 24	24 hours	48 hours	
„ 24	24 hours	48 hours	
„ 24	24 hours	48 hours	
„ 24	24 hours	48 hours	
„ 24	24 hours	48 hours	
„ 26	34 hours	34 hours	
„ 26	24 hours	24 hours	
„ 26		48 hours	
„ 27		24 hours	
„ 30		24 hours	
„ 30		24 hours	
„ 30		24 hours	
„ 30		24 hours	
Dec. 1		48 hours	
„ 3		48 hours	
„ 3		48 hours	

From this Table it appears that in about 24 hours desquamation occurs at the place of injection, but none elsewhere.

The other poisons produced no desquamation as Table XIII. shows.

Table XIII. Frogs poisoned with Aconitia.

Date.	No desquamation in
Dec. 8	48 hours
„ 8	48 hours
„ 8	48 hours
„ 8	48 hours
Extract of Duboisia.	
Jan. 3	40 hours
„ 9	24 hours
Acetate of Strychnia.	
Jan. 8	38 hours
„ 8	38 hours

SUMMARY.

1. Arsenious acid is a poison to all nitrogenous tissues.

2. Whether it is equally poisonous to all nitrogenous tissues, or is especially poisonous to some structures, our experiments do not decide.

3. Whilst it rapidly paralyses the central nervous system, it requires some hours to destroy the conductivity of the motor nerves and muscular irritability.

4. It causes in a few hours separation of the cuticle of the whole body.

CONCERNING THE ACTION OF ACONITIA ON THE NERVOUS AND MUSCULAR SYSTEM OF FROGS.

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Liegeois and Hottot (*Journal de Phys.*, 1861) find that frogs poisoned with aconitia lose sensation whilst voluntary and reflex action remain, and reflex action itself ceases whilst the animal still retains voluntary power. As mechanical arrest of the circulation destroys sensation before voluntary power, and as aconitia is a powerful heart poison, it occurred to us that perhaps aconitia might paralyse by arresting the heart. The following experiments however disprove this surmise, for general and complete paralysis ensue far quicker after aconitia poisoning than after mechanical arrest of the circulation by ligature or extirpation of the heart.

We found, moreover, that the motor nerves and muscles became much sooner paralysed after poisoning by aconitia than after mechanical arrest of the circulation. As aconitia also paralyses the sensory nerves, we conclude that, like potash salts and arsenious acid, aconitia paralyses all nitrogenous tissues, and is a protoplasmic poison.

We used solutions of different strengths, namely, 1, $\frac{1}{3}$, $\frac{1}{6}$, and $\frac{1}{12}$ per cent. In the accompanying Tables we have always noted which preparation was used. The solution, even the weakest, had a slight irritating action. It was always administered under the skin of the back. We then watched the animals till they became completely paralysed, and subsequently, morning, noon, and night, tested the conductibility of the sciatic nerve and the irritability of the muscles with a du Bois induction coil and a one-celled Daniells' battery.

In the first series we performed a comparative test experiment. Thus we simultaneously poisoned one frog, and pithed and pegged another, and cut out its heart, thus testing the effect of arrest of circulation on the nervous and muscular tissues, so that we could compare the effect with that of aconitia on the various structures, and thus ascertain whether aconitia acts simply by arresting the circulation.

Table I. Action of Aconitia on the central nervous system, on conductivity of motor nerves and on muscular irritability.

Date.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Sensation and reflex action destroyed in	Paralysis complete in	Sciatic nerve ceased to conduct in	Muscular irritability ceased in anterior limbs in	Muscular irritability ceased in thighs in	Muscular irritability ceased in lower leg	
Dec. 8	$\frac{1}{100}$	24	$\frac{1}{37056}$		15 min.	4 hours	12 hours	10 hours	10 hours	one per cent. solution employed.
„ 8	$\frac{1}{50}$	26	$\frac{1}{20072}$	3 min.	20 min.	9 hours	10 hours	10 hours	14 hours	
„ 8	$\frac{3}{100}$	29	$\frac{1}{14925}$	3 min.	7 min.	4 hours	14 hours	4 hours	10 hours	
„ 8	$\frac{1}{25}$	29	$\frac{1}{11194}$	2 min.	13 min.	4 hours	10 hours	2 hours	10 hours	
„ 8	$\frac{1}{300}$	30	$\frac{1}{138960}$	2 min.	13 min.	14 hours	26 hours	16 hours	28 hours	$\frac{1}{3}$ per cent. solution
„ 10	$\frac{1}{300}$	46	$\frac{1}{213072}$	5 min.	41 min.	9 hours	28 hours	20 hours	28 hours	$\frac{1}{3}$ per cent. solution
„ 10	$\frac{1}{300}$	39	$\frac{1}{180648}$	5 min.	31 min.	4 hours	14 hours	10 hours	10 hours	$\frac{1}{3}$ per cent. solution
„ 10	$\frac{1}{300}$	37	$\frac{1}{171384}$	6 min.	30 min.	4 hours	14 hours	12 hours	16 hours	$\frac{1}{3}$ per cent. solution
„ 11	$\frac{1}{600}$	28	$\frac{1}{259392}$	4 min.		16 hours	16 hours	20 hours	30 hours	$\frac{1}{6}$ per cent. solution
„ 11	$\frac{1}{600}$	42	$\frac{1}{389088}$	4 min.		12 hours	34 hours	34 hours	40 hours	$\frac{1}{6}$ per cent. solution
			Average	4 min.	21 min.	8 hours	17 hours	13 hours	19 hours	
„ 12	$\frac{1}{1200}$	31	$\frac{1}{574368}$	Became completely paralysed. Voluntary power returned in 32 hours and improved so that the animal could crawl. It died in 120 hours.						$\frac{1}{12}$ per cent. solution
„ 12	$\frac{1}{1200}$	31	$\frac{1}{574368}$	Became completely paralysed in 50 minutes and so remained 72 hours, then gradually regained voluntary power, and died in 120 hours.						
„ 12	$\frac{1}{1200}$	28	$\frac{1}{518784}$	Became nearly completely paralysed then slowly recovered, and had nearly recovered its normal state in 120 hours.						
Comparative experiments with brainless frogs whose heart was cut out.										
Dec. 8						60 hours	48 hours	66 hours	76 hours	
„ 8						60 hours	52 hours	74 hours	90 hours	
					Average	60 hours	50 hours	70 hours	83 hours	

We now give a short general account of the effect of the injection. Immediately after the injection with the stronger solutions the animal suffered from slight shock, and lay quite quiet: in a few seconds or minutes this condition passed away, and the animal hopped about very actively, probably from a slight irritation due to the injected fluid, till it became paralysed. The frogs became very frothy, especially those which moved always most actively. The froth was often so abundant that the frogs lay imbedded in it. Never have we seen an approach to this profuse frothing after poisoning by any other substance.

Soon after the injection, we noticed, in some frogs, a good deal of quivering of the thigh and calf muscles, which lasted only a few minutes, disappearing before the cessation of voluntary movement.

In Table I. we give the results of these experiments.

We shall speak of the action of aconitia, first on the cord and brain, next on the motor nerves, and lastly, on the muscles.

Since after complete paralysis, the motor nerves still readily conduct impressions and the muscles contract on galvanic stimulation, it is evident that the paralysis depends on the influence of the aconitia on the central nervous system. This paralysis of the cord and brain, is it due to the direct influence of the aconitia on these structures? Or does aconitia act on cord and brain solely by arresting the heart, and thus stopping the circulation? The aconitia certainly did not paralyse these structures simply by arresting the circulation: thus in eight experiments it produced complete paralysis on an average in 20 minutes, whilst arrest of the circulation we found, as shewn in Table II., required a much longer interval to establish the condition.

Table II.

Date.	Paralysis complete in
Nov. 29	53 minutes
„ 30	100 minutes
„ 30	102 minutes
Dec. 1	100 minutes
„ 1	74 minutes
Average	86 minutes

Thus it appears that simple arrest of the circulation at the same time of year paralyses the central nervous system in an average time of 86 minutes, whilst aconitia paralyses four times quicker. We therefore are justified in concluding that aconitia does not paralyse the central nervous system by arresting the circulation, but by its direct action on the tissues.

Is the paralysis of the motor nerves and the muscles due to the direct action of aconitia, or to its arrest of the circulation by paralysing the heart? To test this, as we have already explained, we simultaneously poisoned a frog with aconitia, and cut out the heart in a pithed and pegged test frog; then we tested three times daily the nerves and muscles of each frog, and we found that the conductivity of the motor nerves and the muscular irritability ceased much sooner under the poison than under mechanically arrested circulation.

We shall refer first to the motor nerves, and give the averages from Table I.

Average duration of conductivity of sciatic nerve in arrest of circulation	60 hours.
Average duration of conductivity of sciatic nerve in poisoning by aconitia	8 hours.

These results clearly show that aconitia does not paralyse the motor nerves simply by arresting the circulation.

We shall now show by the following averages that aconitia does not paralyse the muscles simply by arresting the heart, and must therefore exert a direct action on the muscles.

Average duration of muscular irritability of anterior limbs in frogs poisoned by aconitia	17 hours.
Average duration of muscular irritability of anterior limbs in frogs with arrest of circulation	50 hours.
Average duration of muscular irritability of thighs in frogs poisoned by aconitia	13 hours.
Average duration of muscular irritability of thighs in frogs with arrest of circulation	70 hours.
Average duration of muscular irritability of lower leg in frogs poisoned by aconitia	19 hours.
Average duration of muscular irritability of lower leg in frogs with arrest of circulation	83 hours.

It thus appears that after simple arrest of the circulation by extirpation of the heart, muscular irritability continues from three to five times longer than after poisoning by aconitia.

We next tested the influence of aconitia on muscular irritability in a different way. Before injecting the poison we tied a ligature tightly round the thigh of the left leg close to the trunk. By this means we effectually protected the left leg from poisoning either through the circulation or by imbibition, and we then tested the muscular irritability in each calf, and give the results in Table III. :

These tables also plainly show the paralysing action of aconitia on muscles. Thus muscular irritability on an average ceased in the

Anterior limbs	10 hours.
Thighs	14 hours.
Right poisoned lower leg	34 hours.
Left ligatured unpoisoned lower leg	77 hours.

This Table shows that muscular irritability persisted in the unpoisoned muscles seven times longer than in the anterior limbs, five times longer than in the thigh, and rather more than twice as long as in the corresponding poisoned muscles of the fellow lower leg.

Aconitia paralyses sensory nerves also, at least so we conclude from the following experiment. On painting the left leg with a solution of aconitia the afferent nerves became completely paralysed, so that strong pinching of this leg neither excited voluntary nor reflex action, while voluntary power remained good.

Aconitia, then, paralyses the nervous centres, the motor and sensory nerves, and the muscles; and we think we may fairly conclude that it paralyses probably all nitrogenous tissues. This effect, we have shown, is not due to the paralysing action of aconitia on the heart. Aconitia therefore must act directly on the tissues; though whether it arrests their functions by combining with the tissues, or simply by arresting oxidation, these experiments do not conclusively establish; but they strongly suggest that it is by combination with the tissues, and not simply by arresting oxidation.

Does aconitia, like chloride of potassium, affect all the tissues in an equal degree, or has it an especial action on certain tissues? If it manifest the same affinity for all tissues, then their functional activity should cease in the order of their activity, and the phenomena should be of the same kind and occur in the same gradation as after simple arrest of the circulation by extirpation of the heart. This, however, does not happen, for, after arrest of the circulation, we find in October, and therefore probably in all the winter months, that sensation continues as long, or almost as long, as voluntary power; but after

Table III. Action of Aconitia on muscular irritability of frogs, with one leg protected from the poison by a ligature applied tightly round the thigh.

Date, 1877.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Paralysis complete in	Muscular irritability continued in				
					Anterior leg.	Right thigh.	Right (poisoned) lower leg.	Left (unpoisoned) lower leg.	
Dec. 18	$\frac{1}{150}$	25	$\frac{1}{57900}$	51 min.	16 hours	16 hours	35 hours	82 hours	Solution 1 in 300
,, 18	$\frac{1}{150}$	37	$\frac{1}{85692}$	35 min.	10 hours	16 hours	50 hours	84 hours	,, ,,
,, 18	$\frac{1}{50}$	19	$\frac{1}{14668}$	12 min.	5 hours	10 hours	16 hours	64 hours	Solution 1 in 100
			Average	33 min.	10 hours	14 hours	34 hours	77 hours	

poisoning by aconitia, even in the winter months, sensation is speedily abolished. Thus, in these experiments, sensation was destroyed on an average in 4 minutes, whilst voluntary power continued 20 minutes. We must conclude therefore that aconitia has an especial action on some portion of the sensory apparatus. Does it act on the afferent nerves, the sensory tract of the cord, or on the sensory perceptive centre of the brain?

According to Liegois and Hottot aconitia first paralyses the sensory perceptive centre, and then the sensory nerves. They base this conclusion, as we believe, on untrustworthy experiments, as we shall now attempt to show.

They find that at a certain stage of the poisoning, sensation is abolished, but voluntary and reflex action remain: for an impression on the skin is conveyed to the cord, and being reflected, produces a reflex act, which is not perceived by the brain, and consequently does not excite voluntary movement. In fact, at this stage, the afferent nerves, as well as the sensory and reflex portion of the cord, are unaffected, and the sensory perceptive centre only is paralysed. Now, in our experiments, though we watched for this peculiar combination, we never witnessed it. In all the experiments much voluntary power remained after complete loss of sensation; but, in every instance, when sensation was destroyed, reflex action was also abolished.

Again, they state that before poisoning the animal, when they ligatured the aorta close to its abdominal bifurcation with the intention of cutting off the poison from the posterior extremities, this procedure failed to prevent the development of anæsthesia; and further, that on tying the artery nearer its origin so as to shut off the circulation from the cord and spinal nerves, yet so as to allow it to reach the brain, then the poison produced loss of sensation as quickly as in poisoned frogs with unligatured vessels.

These refined experiments, we venture to think, are untrustworthy. In the chapter on chloride of potassium, we have shown how readily this substance diffuses itself even in a frog whose heart is cut out before the poisoning; so with regard to aconitia one would suspect that it too could diffuse itself through the tissues independently of the circulation, and therefore in spite of the ligature of the vessels.

To test whether aconitia can diffuse itself through the tissues irrespective of the circulation, we performed the following experiments on three frogs. We made a small incision through the thorax and cut the heart in half (subsequently verifying the operation by a post mortem), thus of course completely arresting the circulation. Into two frogs, weighing respectively 23 and 35 grammes, we injected $\frac{1}{25}$ grain of aconitia (solution 1 in 100) under the skin of the back; the third we left unpoisoned to serve as a test frog.

The poisoned frogs completely lost sensation in two minutes, and in 29 and 24 minutes respectively paralysis was complete, whilst in the unpoisoned frog, at the end of an hour, sensation still continued in every part of the animal and voluntary power was good though somewhat weakened. Now these experiments clearly show how readily aconitia can diffuse itself and reach the central nervous system, without the help of the circulation.

Of course it is not here maintained that ligature of the vessels supplying a limb does not afford any protection, especially when only a small dose of a poison is administered, for numerous observers have witnessed the contrary. But these experiments clearly indicate how readily some poisons, especially when given in large doses, can diffuse themselves.

Nevertheless, whilst we think Liegois and Hottot's experiments are open to objection, we think probably their conclusion that aconitia abolishes sensation by its influence on the brain is correct, and we shall, we hope, also show that the early loss of reflex action is likewise due to the effect of aconitia on the brain. For in unmutilated frogs, aconitia speedily abolishes sensation and reflex action, whilst much voluntary power remains for some time afterwards. In brainless frogs aconitia paralyses reflex action much more slowly, as Table IV. shows.

Table IV. Action of Aconitia on Pithed and Pegged Frogs*.

Date, 1877.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Reflex action completely paralysed in	
Dec. 17	$\frac{1}{300}$	20	$\frac{1}{92640}$	44 min.	Solution 1 in 300
„ 17	$\frac{1}{300}$	28	$\frac{1}{130296}$	27 min.	„ „
„ 17	$\frac{1}{300}$	20	$\frac{1}{92640}$	48 min.	„ „
			Average	39·7	

From these three experiments it appears that in brainless frogs aconitia destroys reflex action in about 40 minutes, whilst our previous experiments show that in unmutilated animals it destroys reflex action

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[*] i.e. Frogs with the spinal cord divided opposite the occipito-atlantal membrane and a peg of wood thrust into the cavity of the skull.

in about 4 minutes. It is obvious therefore that aconitia soon destroys reflex action through its influence on the brain. These experiments show too that aconitia does not paralyse the sensory nerves at an early stage, nor the sensory tracts of the cord, for in brainless frogs these parts, though of course subjected to the action of the poison, still conducted impressions to the reflex portion of the cord; and hence we think it highly probable, that the early loss of sensation as well as of reflex action in unmutilated frogs must be due to the action of aconitia on the brain.

We can now answer the question we put to ourselves at an earlier part of this paper. Has aconitia, like chloride of potassium, an equal action on all the tissues? or does it affect some in a greater degree than others? Now we have seen that aconitia early destroys sensation, through its influence on the brain; early loss of sensation occurs after mechanical arrest of the circulation in the summer months, but not in winter; hence the early loss of sensation after aconitia poisoning in the winter months cannot be due to the powerful action of the poison on the heart. And we must conclude, we think, that probably aconitia has an especial action on the sensory perceptive centre. There are many experiments by other observers showing that aconitia has an especial action on the sensory nerves, and its topical application in man also shows this. Again we must admit that aconitia has an especial action on the heart. Hence we conclude that whilst aconitia is a poison to all tissues it is especially poisonous to some. Now this fact enables us to answer the question,—Does aconitia paralyse by combining with nitrogenous tissues or by arresting oxidation? If it acted by arresting oxidation, then the order of paralysis should be the same as after simple arrest of the circulation, and sensation should not be destroyed early in the winter months; this we have seen is not the case, but aconitia having an especial action on the sensory part of the brain and the heart, and therefore an affinity for these structures, we are therefore led to conclude that aconitia probably acts not by arresting oxidation, but by an affinity for all nitrogenous tissues, especially for the sensory centre of the brain and the heart.

How does aconitia affect the heart? Böhm and Wartmann find that it first depresses and then paralyses the vagi, and that with complete paralysis the muscular irritability of the heart is destroyed. The preceding experiments lead us to suggest that, being a protoplasmic poison, aconitia not only paralyses the vagi and the muscular tissue, but likewise all the nervous structures connected with the heart.

THE ACTION OF TARTAR EMETIC, HYDROCYANIC ACID AND VERATRIA ON THE ANIMAL BODY.

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Having shown that potash salts, arsenious acid, and aconitia are powerful protoplasmic poisons, destroying function in nitrogenous tissues, it occurred to us that perhaps all poisons might prove to be protoplasmic poisons, but that some, whilst highly destructive to the functions of certain tissues, might be only in a slight degree destructive to the functions of other tissues. In this paper we proceed to follow out this supposition, and we commence with tartar emetic.

In the present series of experiments, as in our previous series, we injected the poison under the skin of the frog's back. To ascertain whether tartar emetic affects the tissues directly, or only by arresting the heart, we prepared some test frogs, by making an incision through the heart and so arresting the circulation.

Then we tested the conductivity of the motor nerves and the muscular irritability by a one-celled du Bois-Reymond induction coil.

The results are shown in Table I.

In these two experiments the frogs became completely paralysed in 114 and 113 minutes respectively. As after complete paralysis the motor nerves readily conducted impressions to the muscles and the muscles contracted energetically on the direct application of the electrodes, it is evident that the general paralysis depended on paralysis of the central nervous system.

Is this paralysis of the central nervous system due to the direct action of the tartar emetic, or does it depend on arrest of the heart? The paralysis is clearly due to the direct action of the poison on the central nervous system, for in one frog the heart beat visibly through the abdominal walls till twenty minutes before complete paralysis; and in the second frog, after complete paralysis, the heart beat freely at twenty per minute. Now in the two test frogs, with mechanical

Table I. The Action of Tartar Emetic on the Nervous and Muscular System of Frogs.

Date, 1878.	Dose in grains.	Weight of animal in grammes.	Proportionate dose.	Paralysis complete in	Heart continued beating	Sciatic nerves paralysed in	Muscular contractility ceased				Strength of Solution.
							In anterior limbs.	In back.	In thigh.	In lower leg muscles.	
Mar. 18	$\frac{1}{5}$	40		114 minutes	94 minutes	7 hours	4 hours	4 hours	7 hours	10 hours	1 in 20
,, 18	$\frac{1}{5}$	40		132 minutes	more than 132 minutes *	7 hours	7 hours	7 hours	7 hours	10 hours	1 in 20
			Average	123 minutes		7 hours	5·5 hours	5·5 hours	7 hours	10 hours	
Test Frogs. Heart incised.											
Mar. 19				76 minutes		17 hours	17 hours	18 hours	20 hours	21 hours	
,, 19				75 minutes		27 hours	20 hours	33 hours	34 hours	34 hours	
			Average	75·5 minutes		22 hours	18·5 hours	25·5 hours	27 hours	27·5 hours	

* When the paralysis was complete the heart still beat visibly through the walls at 20 per minute.

Table II. Action of Tartar Emetic on Muscular Irritability.

Date, 1878.	Dose in grains.	Weight of animal in grammes.	Proportionate dose.	Muscular irritability ceased in				Strength of solution.
				Anterior limbs.	Back.	Thigh.	Lower leg muscles.	
Jan. 22	$\frac{1}{4}$	34		10 hours	10 hours	20 hours	16 hours	1 in 20
,, 22	$\frac{1}{4}$	21		10 hours	10 hours	12 hours	12 hours	,,
,, 23	$\frac{1}{5}$	19		9 hours	14 hours	9 hours	14 hours	,,
,, 23	$\frac{1}{10}$	25		20 hours	28 hours	40 hours	40 hours	,,
,, 24	$\frac{1}{5}$	25		18 hours	18 hours	28 hours	36 hours	,,
,, 24	$\frac{1}{5}$	22		20 hours		20 hours	20 hours	,,
			Average	14·5 hours	16 hours	21·5 hours	23 hours	
			Test brainless frogs with heart cut through.					
Jan. 22				86 hours	58 hours	84 hours	110 hours	
,, 23				64 hours	44 hours	64 hours	84 hours	
			Average	75 hours	51 hours	74 hours	97 hours	

arrest of the circulation, paralysis was not complete till 76 and 75 minutes respectively. Tartar emetic is therefore a paralyser of the central nervous system.

Tartar emetic is also a direct paralyser of the motor nerves; for the sciatic nerve of the frogs treated with tartar emetic ceased to conduct impressions much earlier than the sciatic nerve of frogs with mechanical arrest of the circulation, as the following averages taken from the preceding Table show. Sciatic nerve ceased to conduct in

Frogs poisoned with tartar emetic	7 hours.
Frogs with mechanical arrest of the circulation	22 hours.

We shall now show that tartar emetic is a muscle poison. We injected the solution under the back of a frog, and at the same time we prepared a test frog by dividing the heart and so arresting the circulation. The following averages (Tables III. and IV.) which we take from Tables I. and II. show that muscular contractility ceased much sooner in the frogs treated with tartar emetic than in the frogs with mechanical arrest of the circulation, proving that tartar emetic is a direct muscle poison and that it does not destroy contractility by arrest of the heart.

Table III. Resumé of Table I.

Muscular irritability ceased in	Frogs poisoned with tartar emetic.	Frogs with mechanical arrest of circulation.
Anterior limbs	5·5 hours	18·5 hours
Back	5·5 hours	26·5 hours
Thighs	7 hours	27 hours
Lower leg muscles	10 hours	27 hours

Table IV. Resumé of Table II.

Muscular irritability ceased in	Frogs poisoned with tartar emetic.	Frogs with mechanical arrest of circulation.
Anterior limbs	14·5 hours	75 hours
Back	16 hours	51 hours
Thighs	21 hours	74 hours
Lower leg muscles	23 hours	97 hours

Tartar emetic is a poison not only to voluntary muscular tissue, but like aconitia, when administered hypodermically, it is a poison also to the muscular substance of the heart and destroys its contractility.

Tartar emetic paralyses sensation; through what part of the sensory apparatus is uncertain, though Radziejewski believes it affects the sensory part of the cord.

The topical application to the skin of the frog does not seem to affect sensation, so at least we conclude from two experiments. We immersed the left leg of a frog two minutes and the same limb of a second frog five minutes in a solution (1 in 20) of tartar emetic, apparently without affecting sensation, or only in so slight a degree that it was difficult to make sure there was any loss of sensation.

We have thus shown that tartar emetic paralyses the central nervous system, the motor nerves, the muscles, and destroys sensation, and therefore we are led to conclude that probably tartar emetic is a protoplasmic poison, destroying function in all nitrogenous tissue. Our experiments, however, fail to show whether it manifests for all nitrogenous tissues an equal affinity, or whether it has a special action on some.

We suggest that, as in the case of potash salts, arsenious acid, and aconitia, tartar emetic weakens or paralyses the heart, through its action on all the tissues, ganglia, nerves, and muscular substance.

We noticed a peculiar effect of tartar emetic on the skin. In a few hours the cuticle of a poisoned frog became softened and reduced to a jelly-like condition, too soft to be stripped off, though it could be easily scraped off every part of the body. Even small doses soon produce this remarkable effect, for in one experiment the skin, after a small dose, became in this condition even whilst the animal could still crawl pretty well.

Kölliker's experiments show that hydrocyanic acid is a powerful protoplasmic poison, destructive to the function of all nitrogenous tissue. He finds* that after poisoning with prussic acid, the nerves no longer conduct impressions and the muscles no longer respond to the application of the electrodes. The sensory nerves are also paralysed. Kölliker concludes that prussic acid paralyses, first the brain, next the cord, then the motor nerves. It destroys the contractility of the heart even on the direct application of the electrodes to the muscular substance.

We suggest that veratria too is a protoplasmic poison. It is undoubtedly a powerful muscle poison, peculiarly modifying the character of the contraction. Its powerful paralysing action on muscles renders it difficult to test its action on the nervous centres and motor nerves, but the experiments of Bezold and Hirt* prove that veratria paralyses the motor nerves, and clinical experience shows that its application to the skin paralyses the terminations of the sensory nerves. Veratria also paralyses the heart muscle.

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* Virchow's *Archiv*, Bd. x.

Veratria therefore paralyses voluntary muscular fibres, the heart muscle, the motor and sensory nerves; and we venture to suggest, though we are unable to adduce any experimental proof, that it probably paralyses the central nervous system, though perhaps only to a slight degree. Veratria in fact is a protoplasmic poison.

Potash salts, arsenious acid, aconitia, tartar emetic, hydrocyanic acid, are then powerfully poisonous to the central nervous system, to motor and sensory nerves, and to muscles. Experiment shows too that veratria is also poisonous to muscle and nerves, but there are no experiments to prove its poisonous action on the central nervous system. It seems highly feasible that all these substances are powerful protoplasmic poisons, depressing or destroying function in all nitrogenous tissue.

All are cardiac depressants, and we suggest that their action does not depend on their affecting a single structure of the heart, but that they paralyse the ganglia, their nerves, and the cardiac muscle itself; further, we deem it highly probable that the action of these agents on the heart does not entirely explain their depressing effect; they depress in fact also by their poisonous action on the central nervous system and on the motor nerves and muscles.

We have suggested that potash has an equal action on all nitrogenous tissue, but we need hardly say, that whilst the remaining substances must be considered poisonous to nitrogenous tissue, we do not mean that they are equally poisonous to each structure; no doubt some exert a special poisonous action on one tissue, as, for instance, veratria on muscle.

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* *Untersuch. aus dem Labor. zu Wurzburg,* Heft 1.

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ON PITURI.

By SYDNEY RINGER, M.D., and WILLIAM MURRELL, M.R.C.P., *Lecturer on Practical Physiology at the Westminster School of Medicine, and Assistant Physician to the Royal Hospital for Diseases of the Chest.*

QUITE recently a student of University College, London, whose name we have unfortunately forgotten, gave us a small packet containing a few twigs and broken leaves of the powerful and interesting drug Pituri. These we placed in Mr Gerrard's hands, and he kindly made first an extract from which he obtained a minute quantity of an alkaloid, and with this he made a solution containing one part of the alkaloid to twenty of water.

Baron Mueller, from an examination of the leaves of pituri, is of opinion that it is derived from Duboisia Hopwoodii. Pituri is found growing in desert scrubs from the Darling River and Barcoote to West Australia. The natives, it is said to fortify themselves during their long foot marches, chew the leaves for the same purpose as Cocoa leaves are used in Bolivia. Dr G. Bennett in the New South Wales Medical Gazette, May, 1873, says Pituri is a stimulating narcotic and is used by the natives of New South Wales in like manner as the Betel of the East. It seems to be a substitute for tobacco.

It is generally met with in the form of dry leaves, usually so pulverized that their character cannot be made out.

The use of pituri is confined to the men of a tribe called Mallutha. Before any serious undertaking, they chew these dried leaves, using about a tea-spoonful. A few twigs are burnt and the ashes mixed with the leaves. After a slight mastication the bolus is placed behind the ear (to increase it is supposed its strength), to be again chewed from time to time, the whole being at last swallowed. The native after this process is in a sufficiently courageous state either to transact business or to fight. When indulged in to excess, it is said to induce a condition of infuriation. In persons not accustomed to its use pituri causes severe headache.

In a paper read before the Queensland Philosophical Society in March, 1872, Dr Bancroft described some experiments made with an infusion, and attributes to the drug the following effects:

I. Period of preliminary excitement from apparent loss of inhibitory power of the cerebrum, attended in cats and dogs with rapid respiration, vomiting, and profuse secretion of saliva.
II. Irregular muscular action followed by general convulsions.
III. Paralysis of the respiratory function of the medulla.
IV. Death, or
V. Sighing respiration at long intervals.
VI. Rapid respiration and returning consciousness.
VII. Normal respiration and general torpidity not unattended with danger to life.

We began our investigations on August 16, by injecting under the skin of a small cat one minim of the 1 in 20 solution. In three minutes the cat staggered while walking. In seven minutes the animal could walk though badly; it lay down on the table. In eight minutes it was still weaker, so that when lying down it let its head rest on the table. The breathing was 32 per minute and laboured, but the mucous membrane of the mouth was not at all livid. In nine minutes we observed muscular cutaneous twitching. In thirteen minutes it seemed sleepy, but could see, hear, and feel, distinctly. In fifty minutes it could still walk but rolled a good deal, and the muscular weakness was shown by the ease with which we could open its mouth. The mouth, which at first became a little moister, was now decidedly dry. Respirations 20, rather deep. In 25 minutes the cat began to improve, though the mouth remained a little dry; and in 30 minutes, with the exception of a little dullness, it had recovered. In 34 minutes we again injected two minims of the solution. In one minute the breathing rose from 28 to 36 per minute and became laboured. In four minutes the cat vomited violently and passed water. It became also weak and staggered a little. In eight minutes the respirations were 44, and superficial. The cat walked badly and the mouth was a little dry. In twelve minutes it seemed sleepy. In twenty minutes it still staggered on walking. The respirations had fallen to 28. The pupils were widely dilated, and the mouth was decidedly though not extremely dry, and the cat was drowsy. In 46 minutes the pupils were still dilated, and the mouth rather dry, though in other respects the animal had nearly recovered, but remained a little dull.

In this experiment the drug produced hurried and laborious breathing. Much muscular weakness with slight twitching. Slight dryness of the mouth. Slight drowsiness and wide dilatation of the pupils. We were struck by the rapidity of the onset of the symptoms and their short duration, showing that the alkaloid passes readily through animal membranes and is therefore readily absorbed and readily destroyed or excreted, probably excreted.

We then applied a small quantity of the alkaloid solution to the right eye of a cat. In 40 minutes the right pupil became rather larger than the left, and in 45 minutes twice the size of the left; and in six hours the right pupil was three times as large as the left. In twelve hours the pupils became equal. Thus the drug widely dilated the pupil, but the dilatation lasted less than twelve hours.

We next injected six minims of the 1 in 20 solution under the skin of a moderate-sized cat. In one minute it staggered much in walking, and the breathing became panting. In eight minutes the movements were quite stiff, the legs seemed without joints and the cat seemed walking with sticks not legs. The limbs were evidently rigid from strong muscular contraction. The respirations were 130 in the minute, and the mouth was covered with foam, a few drops of saliva dropping on to the table. The mouth was very moist. In nine and again in ten minutes the cat vomited, and soon after passed water and defecated. The ears twitched a good deal and the third eye-lid was partially closed. In this state the animal continued till thirteen minutes after the injection, when all parts of the body twitched violently, the twitchings being brought on by tapping the animal or gently pinching the skin, or even tapping the table or blowing on the animal. The movements were not like tetanus, but consisted of numerous convulsive jerks of the limbs and trunk, the legs not being extended as in tetanus, nor the body curved. In fifteen minutes the animal lay on its side panting, the breathing being very superficial; it looked as if it were dying. In eighteen minutes the respirations were 92 the minute, and as before seemed almost entirely diaphragmatic. In other respects the cat seemed in the same state. In twenty minutes the pupils became widely dilated, the mouth continued very moist and was covered with foam. In 25 minutes the animal began to improve, having regained a little strength, and could raise its head. In 26 minutes the respirations were 104, still diaphragmatic, but occasionally the cat took a deep costal inspiration. In 32 minutes the twitchings and jerks began to grow less. In 38 minutes there was considerable improvement, for the cat could move better. The mouth was now quite dry. In 42 minutes the respirations were 68, and the pupils were almost fully dilated. The cat could now sit up, but the jerkings, though less persistent, continued. In 50 minutes the breathing was more costal and the mouth was moister. The cat could now sit up and licked itself. There was however still much muscular weakness. In 68 minutes it still staggered whilst walking, and some twitchings occurred on tapping it. The pupils were dilated and the mouth dry. In seven hours the animal had nearly recovered. It was not at all weak, but slight twitching could be induced on tapping the body or head with the forceps. The pupils were natural and the mouth was moist. Next day the cat had quite recovered.

In this experiment we had these additional symptoms: salivation followed by dryness of the mouth, and strong tonic rigidities of the limbs as well as powerful clonic twitching of all parts of the animal.

We next experimented to ascertain if there was any antagonism between pituri and muscarin, similar to that between atropia and muscarin, on the frog's heart. We divided the cord of the frog opposite the occipito-atlantoid membrane, and passed a wooden peg into the cavity of the skull, destroying the brain. We then opened the chest and exposed the heart. We made two experiments. In the first experiment, in two minutes after the application of a minute quantity of extract of muscaria to the auricles, the heart had almost stopped, and only contracted very slightly at long intervals. We then applied a small quantity of the solution of the pituri alkaloid, and in half a minute the heart beat strongly at 28 per minute; and in two minutes and a half, at 36 a minute, and for a long time after it continued to beat strongly.

In our second experiment, after the application of the extract of muscaria, the heart completely stopped in wide diastole. We then applied the solution of pituri alkaloid, and in half a minute the auricles began to pulsate very feebly. In two minutes and a half the ventricle began to contract, and in three minutes the heart was contracting strongly at 48 per minute, and so continued for an hour or longer, beating well when we discontinued our observations.

These experiments show that pituri, like atropia, is a powerful antidote to the action of muscarin on the heart.

We next applied the pituri alkaloid to an exposed frog's heart, but found that it produced no obvious change, unless it somewhat strengthened the contractions.

At this point, our stock of alkaloid became exhausted, and we were thus compelled to discontinue our investigations.

As we had already shown that *Duboisia myoporoides* acts in most respects like atropia, the fact of pituri being a solanaceous plant, and moreover a Duboisia, led us to expect that pituri would manifest the properties of atropia, and our experiments confirm this conclusion. We find that pituri causes drowsiness, dilates the pupil, dries the mouth, produces general weakness with convulsive twitchings, and antagonises the action of muscarin on the heart. It possesses, however, two properties distinct from those of atropia; it produces sickness and increases the salivary secretion, in large doses copiously, before it dries the mouth. We may here observe in passing that large doses of hyoscyamia may induce nausea and sickness. Even in the properties in which pituri resembles, it still differs from, atropia. For atropia chiefly dries the mouth, dilates the pupil, and these effects endure many hours, indeed with respect to the dilatation of the pupil even days; whilst only large doses produce drowsiness, general weakness, and twitchings; or quicken the respirations. On the other hand with pituri the earliest effect is manifested on the breathing, which becomes quick and shallow; then follows general weakness, and after large doses, severe muscular twitchings, whilst the pupil is far less dilated, and the dilatation lasts for a shorter time than after atropia. Moreover pituri dries the mouth far less than atropia, and the dryness is preceded by an increased salivary secretion.

It appears, therefore, that whilst the alkaloid of pituri retains the properties of solanaceous plants it differs somewhat from atropia. We suggest that it may be atropia slightly modified, so that its effects on some of the animal structures are lessened, whilst on other structures its effects are increased; that in fact its influence on secretion and on the pupil is lessened, whilst its effect in producing general weakness and twitchings, and its influence on respiration, are greatly intensified.

This modification of atropia, this pituri alkaloid, is in its physiological effects, and therefore probably in chemical constitution, allied to certain other alkaloids. This alliance will be better seen if we give first a summary of the effects of pituri, and then of the alkaloids to which it seems affiliated.

Pituri is—

1. Slightly narcotic.

II. It first salivates and then dries the mouth.

III. It dilates the pupil when administered internally or applied topically to the eye.

IV. It is a powerful respiratory poison, and, according to Dr Bancroft, it kills in this way—a conclusion which our experiments confirm, for our cat nearly perished through the action of the poison on respiration.

V. It produces general weakness.

VI. It produces violent twitchings of the whole body.

Gelsemium—

I. Is slightly narcotic.

II. Salivates cats.

III. Administered internally it contracts: applied topically it first contracts and then dilates the pupil.

IV. It is a powerful respiratory poison.

V. It produces general paralysis.

VI. It paralyses and tetanizes frogs.

Jaborandi—

I. Is slightly narcotic.

II. It salivates profusely.

III. Internally administered, or topically applied, it contracts the pupil.

IV. It does not affect respiration.

V. It produces muscular tremblings.

VI. It paralyses and tetanizes frogs.

Through jaborandi pituri becomes allied to muscarin, which possesses the same actions as jaborandi, except that muscarin administered internally contracts, but topically applied dilates the pupil.

By means of pituri, therefore, we group into alliance alkaloids apparently so dissimilar as atropia and jaborandi, and atropia and muscarin, two alkaloids which in most of their effects are antagonistic to those of atropia.

The idea naturally arises that by slight modifications of an alkaloid its properties may become greatly changed and even reversed, without a corresponding change in its chemical constitution. Some experiments * made by one of us on the daffodil and snowdrop and some other amaryllidaceous plants tend to support this hypothesis. The daffodil alkaloid from the bulb of the flowering plant dried the mouth, checked perspi-

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* An account of these will be published in the next number of this Journal.

ration, dilated the pupil, and acted in most respects like atropia, but strange to say, the alkaloid derived from bulbs gathered after the plants had finished flowering, profusely salivated, whilst it still retained its power to dilate the pupil. Here it would seem that by some modification of the alkaloid its action on the salivary secretion becomes reversed, whilst its action on the pupil becomes weaker.

What may be the nature of the change in the composition of the alkaloid, our investigations in no way help us to determine. It may be similar to that change whereby, as Dr Fraser has pointed out, strychnia when converted into an ethyl compound loses its tetanizing action, and becomes a paralyser, though without undergoing change in its chemical constitution, for ethyl-strychnium gives the same chemical reactions as strychnia itself.

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A FEW FURTHER EXPERIMENTS WITH PITURIA.

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In a previous communication in this *Journal* (Vol. I. No. 5) we showed that pituria, the alkaloid of pituri, a solanaceous plant, and belonging to the subdivision Duboisia, manifests many of the properties of atropia. Pituria causes drowsiness, dilates the pupil, produces general weakness with convulsive twitchings, and antagonizes the action of muscarin on the heart. It possesses, however, two properties distinct from those of atropia; it produces sickness and increases the salivary secretion in large doses copiously. Even in the properties in which pituri resembles atropia there are still points of difference. For atropia chiefly dries the mouth and dilates the pupil, and these effects endure many hours, indeed the dilatation of the pupil may last even days, whilst only large doses produce drowsiness, general weakness and twitchings, or quicken the respirations. On the other hand, the earliest effect of pituria is manifested on the breathing, which becomes quick and shallow; then follow general weakness, and, after large doses, severe muscular twitchings, whilst the pupil undergoes far less dilatation, and that lasting a shorter time than after atropia.

Further investigations on the human subject[1] have for the most part confirmed these observations and increased our knowledge of the drug. We find that $\frac{1}{10}$ to $\frac{1}{8}$ gr. of nitrate of pituria given hypodermically produces general weakness, slight rhythmic tremor, quickens the pulse and respirations, and produces general perspiration, in some cases slightly, in others abundant. All these symptoms last a short time only, disappearing in half an hour, or even in a shorter time. This dose never dried the mouth nor produced salivation. Moreover, instead of dilating the pupils it always produced slight contraction; therefore we conclude, that when given internally small doses contract but large doses dilate the pupil; and probably, though we have no observations to prove this, large doses first contract and then dilate the pupil.

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[1] *Lancet*, March 1, 1879.

In our previous communication we showed that pituri antagonizes the action of muscarin on the heart. We now record some experiments showing that it antagonizes the action of pilocarpine on the heart[1].

The following experiments were made in January on the exposed hearts of brainless frogs. We used a solution of nitrate of pituria, and in the first experiments a 2 p. c. solution of nitrate of pilocarpine, and afterwards a 10 p. c. solution.

Exp. 1. To a heart, beating at 52 per minute, we applied a small quantity of a 2 p. c. solution of nitrate of pilocarpine, and in half a minute the heart stopped. Soon after we applied a small quantity of a 1 p. c. solution of nitrate of pituria, and in 15 seconds the heart begun to beat again, and in one minute beat strongly at 24 per minute: in three minutes at 32, and soon after at 40. In 34 minutes the beats became very feeble at 32 per minute, and we again applied a little of the pituria solution, when the beats rose to 40 and became much stronger, so remaining during the next quarter of an hour, when we suspended our experiments. Seven hours afterwards the heart beat fairly well at 22 per minute.

In the three following experiments the effect of the pilocarpine was rather different. As sometimes happens with muscarin, the pilocarpine did not greatly slow, but greatly weakened the heart's contractions, and the pituria increased both their frequency and especially their strength.

Exp. 2. With the heart's contractions at 44 per minute, we applied some of the pilocarpine solution. In seven minutes the beats fell to 20; in the next twenty minutes they rose to 28; but the contractions became extremely feeble. We then applied the pituria solution, and in one minute the beats became stronger and rose to 40, and in two minutes to 44 good beats per minute. In ten minutes, whilst still beating 44, the contractions became very feeble, but on again applying some pituria the contractions at once grew stronger, though in six minutes they again grew feeble.

Exp. 3. Whilst the heart beat well at 48 we applied the pilocarpine solution, and in nine minutes the pulsations fell to 28 and became feeble; in twenty-five minutes, whilst at 32, they became very feeble. Then on applying pituria the beats in half a minute grew much stronger, rising in six minutes to 40. In nine minutes the heart beat strongly at 48 per minute, and seven hours afterwards it beat fairly well at 28.

Exp. 4. To a heart beating at 52 we applied pilocarpine and the pulsations at once fell to 24, but in the course of fifteen minutes they rose to 36 and became very feeble. We then applied nitrate of pituria, and at once the contractions became much stronger, the cavities contracting fairly well at 34. In eight minutes the beats rose to 40. Four hours after the application of the pituria the heart beat fairly well at 20.

[1] The pituri we used was given us by Mr MacAlister, through Mr E. A. Clare, of the Queensland section of the Paris Exhibition. It was grown in the Botanic Gardens at Brisbane by Mr Hill. Mr Gerrard extracted the alkaloid and prepared the nitrate for us.

Exp. 5. We applied a little of a 10 p. c. solution of nitrate of pilocarpine to a heart beating at 40 per minute. The heart stopped almost immediately in wide diastole. After two minutes of complete arrest a little pituria was applied about every half minute. During the next eight minutes the heart gave a strong contraction, and after eight minutes it suddenly commenced beating strongly at 30 per minute, and so continued during the time we watched it, that is the next ten minutes.

Antagonism between pituria and pilocarpine on the frog's heart.

Date.	Decrease after application of pilocarpine.	Loss of strength after application of pilocarpine.	Increased frequency after application of pituria.	Increased strength after application of pituria.	Contractions continued more than
Jan. 1	Heart stopped	Heart stopped	40 beats	became strong	seven hours
„ 1	16 beats	extremely feeble	12 beats	„	
„ 1	16 beats	„	12 beats	„	seven hours
„ 2	16 beats	„	4 beats	„	four hours
„ 2	Heart stopped	Heart stopped	30 beats	„	

We see then that pituria will quicken and greatly strengthen a heart slowed and much weakened by pilocarpine, and even restore the contractions to a heart arrested by pilocarpine. In respect therefore to its antagonism to muscarin and pilocarpine, the solanaceous plant pituri acts like the alkaloids from other solanaceous plants, for instance atropia and duboisia.

As pituria, like atropia, antagonizes the action of muscarin and pilocarpine on the frog's heart, we were anxious to know if pituria, like atropia, paralyses the inhibitory apparatus of the heart. Mr Waters, of the Physiological Laboratory at Cambridge, kindly performed this experiment for us, and finds that pituria has by itself no direct effect on the number or force of the heart's contractions, but so affects the vagi that electric stimulation of them no longer slows or arrests the heart but increases the frequency of the beats.

Pituri therefore is more closely allied to tobacco, as Baron Müller has suggested, than to any other solanaceous plant, for tobacco contracts the pupil, topically applied to the eye or when administered by the stomach. It also causes salivation and perspiration, great weakness, and sometimes violent muscular tremblings.

ON THE ANTAGONISM BETWEEN PILOCARPINE AND EXTRACT OF AMANITA MUSCARIA.

By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London,* and WILLIAM MURRELL, M.D., M.R.C.P., *Lecturer on Practical Physiology at Westminster Hospital School of Medicine, Assistant Physician to the Royal Hospital for Diseases of the Chest.*

We are anxious at the outset to acknowledge our indebtedness to Mr E. A. Morshead, who largely assisted us in the experiments performed in July.

Pituri, as we have shown[1], excites salivation and perspiration, corresponding in this respect to muscarin and pilocarpine. Pilocarpine and muscarin, however slow, weaken and even arrest the heart, whilst, if we may assume this from one experiment, pituri, topically applied, has little or no action on the cardiac contractions. Pituri moreover antagonizes the action of muscarin and pilocarpine on the heart; thus we have a sweater and a salivator antagonizing the action on the heart of substances which likewise cause salivation and perspiration.

It occurred to us that possibly muscarin and pilocarpine, though they affect the heart in a similar manner, might antagonize each other's effects, and we now record some experiments designed to test the validity of this conjecture.

In all these experiments we first killed the frog by dividing the spinal cord opposite the occipito-atlantoid membrane, and destroying the brain by thrusting a peg into the skull. We then, without dividing the sternum, exposed the heart and cut through the pericardium.

We used an alcoholic extract of amanita muscaria, and a solution of nitrate of pilocarpine, in some cases a two per cent., in others a ten per cent. solution.

Jan. 3. To a heart beating 44 per minute we applied a small quantity of extract of muscaria. In four minutes the heart stopped, widely distended with blood. Three quarters of a minute afterwards we applied some 10 p. c.

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[1] *Journal of Phys.*, 1878, p. 377, and *Lancet*, 1879.

solution of nitrate of pilocarpine, when the heart almost immediately began to contract, and in one minute it beat well at 12 per minute, and in forty-five minutes it beat 24. The strength of the contractions then again grew weaker, till in thirty-eight minutes it beat very feebly at 20. Then we applied a little atropia, and at once the beats improved much in strength, rose to 24, and in twenty-two minutes the beats were good at 40 per minute, and six hours later the heart still contracted well at 36 per minute.

On the same day we repeated the experiment. To the exposed heart we applied a small quantity of muscarin, and in eight minutes the pulsations fell from 40 to between 4 and 6 per minute. We then applied a little of the 10 p. c. solution of nitrate of pilocarpine, and in half a minute the pulsations rose to 28 very good beats; in 13 minutes the pulsations began to grow feeble, and in 44 minutes dropped to 12 per minute, and were so feeble as to be scarcely visible. Five hours afterwards the heart was motionless, flaccid, and contained a little blood, having we felt no doubt long ceased to beat, since general reflex action had quite ceased; still we determined to apply a little of the 1 p. c. solution of nitrate of pituria, not for a moment expecting that it could restore the lost cardiac action: but to our astonishment in six minutes the heart began to contract; in ten minutes it beat faintly at 16 per minute; in twelve minutes the contractions were much stronger; in fourteen minutes it beat fairly well at 18, and an hour and a quarter at 20 per minute.

Last summer one of us, in conjunction with Mr E. A. Morshead, made a series of observations regarding the antagonism of pilocarpine for muscarin, making use of a solution of muscarin containing one part of alcoholic extract of amanita muscaria in four parts of water, and one part of nitrate of pilocarpine in twelve parts of water.

July 10. Whilst the heart beat at 54 we applied the muscarin solution freely, which at once slowed the heart to 46, and gradually in the course of thirteen minutes the beats fell to 32 and grew feebler, so that at the expiration of this time the heart contracted hardly at all and remained widely distended. Then we applied some of the pilocarpine solution, when the contractions at once very much improved and became fairly strong, with unaltered frequency for ten minutes, and then rose to 44. The heart continued to beat fairly well at 44 for five hours; in ten hours afterwards it beat feebly at 44, and in twenty-four hours it had stopped, firmly contracted.

July 10. Muscarin solution applied to the heart beating at 58 at once weakened the contractions and reduced them to 34, and this number gradually fell till in eleven minutes it had dropped to 22, when the auricles had ceased to contract and the ventricle contracted very slightly indeed. At this stage we freely applied the pilocarpine solution, and after a pause of some seconds the ventricle at once commenced work fairly well at 28, and in three minutes both auricles and ventricle contracted firmly at 34; in fact as strongly as before the application of the muscarin, and so continued during nine minutes. On our return in two hours we found the heart stopped in wide diastole.

July 10. To a heart beating at 46 we applied some muscarin solution and the pulsation fell in two minutes to 28 and almost stopped, the ventricle, widely distended with blood, alone contracting in the slightest degree, so that only a slight quiver of its walls could be seen. We then applied pilocarpine. In two minutes the ventricle contracted strongly; in six both auricles and ventricle contracted well at 30; the contractions then rose to 44; ten hours later the heart beat well at 44, and even twenty-four hours later it still contracted slightly.

July 11. To a heart beating at 54 we applied the muscarin solution, and in six minutes the pulsation ceased. Then we applied some pilocarpine solution. In one minute pulsation recommenced, and in two minutes rose to 24. In seven minutes we reapplied the pilocarpine, and in thirteen minutes from the second application the heart beat well at 24; the pulsation then grew slower and feebler, so in fifty-three minutes after the first application we again applied pilocarpine, but without strengthening the heart, which continued to beat at 12 per minute. We then reapplied some muscarin and subsequently some more pilocarpine, but the heart never improved, and in fifty-eight minutes from the first application of the pilocarpine only the auricle beat very faintly.

July 11. To the heart beating at 44 we applied some muscarin solution. The pulsations fell to 30 and the auricle stopped, whilst so feeble was the contraction of the ventricle that apparently it was unable to propel any blood. Then we applied pilocarpine and the pulsations rose to 34, and both auricle and ventricle contracted fairly well. In twenty-seven minutes the contractions became feebler, and on returning two hours after the heart had stopped, widely distended with blood.

July 11. To a heart beating at 46 we applied some muscarin. In four minutes the heart stopped, widely distended with blood. We then applied some pilocarpine solution, and for the next six minutes no contractions occurred; in nine minutes the auricles contracted slightly; in fourteen minutes we reapplied pilocarpine, still only the auricles contracted, and fairly well at 32.

It occurred to us that possibly the revivifying effect of pilocarpine applied to the heart previously weakened or arrested by muscarin might be simply a physical result caused by the application of the solution, and not a specific effect of the pilocarpine, and we resolved to make an experiment to ascertain whether this might be a correct surmise.

On Jan. 3 we applied a little muscarin to a brainless frog's heart beating well at 32 per minute. During the ensuing five minutes the beats fell to 4, but were fairly good. We then washed the heart well for five minutes with a 1 p. c. saline solution, but without in the slightest degree quickening or strengthening its beats. Then we applied a little (1 in 10) pilocarpine solution. In two minutes the heart grew much stronger and beat 16, but in four minutes it again grew weaker and fell to 8. Eight minutes later we again applied pilocarpine, and in six minutes the heart, pulsating well, rose to 16. It continued in the same condition thirty-two minutes after the second application of pilocarpine and then grew weaker, though still it continued beating at 16. We then washed the heart well for three minutes with saline solution without affecting either its force or frequency, and an hour after the second pilocarpine application, with the heart at 16, we added a little 1 p. c. solution of sulphate of atropia, when the pulsations improved and rose to 24.

This experiment confirms the preceding, and shows that pilocarpine antagonizes for a short time the effect of muscarin, and that this revival of the heart's action is not due to the mechanical effect of the pilocarpine solution, for a saline solution applied freely for five minutes produced no effect.

Table I.

Date.	After the application of muscarin the pulsations fell	After the application of muscarin the pulsations became	After the application of pilocarpine the pulsations rose	After the application of pilocarpine the pulsations became	The pulsations continued	Pulsations had ceased in
Jan. 3	stopped	stopped	24 beats	good		
,, 3	36 beats	,,	24 beats	good		5 hours
,, 3	28 beats	remained fairly good	12 beats	much stronger		
July 10	22 beats	almost stopped	12 beats	much stronger	10 hours	
,, 10	36 beats	ventricle alone contracted and very feebly	12 beats	good		2 hours
,, 10	18 beats	almost stopped, auricle quite stopped	16 beats	good	24 hours	
,, 11	54 beats	stopped	24 beats	good		1 hour
,, 11	14 beats	auricle stopped and ventricle contracted very feebly	4 beats	fairly good		2 hours
,, 11	stopped	stopped	auricles only at 32	feeble and only in auricle		1 hour

A glance at Table I., in which we have summarised the results of the foregoing experiments, shows at once that pilocarpine in a great measure antagonizes the action of muscarin on the heart; that pilocarpine restores vigorous contractions to an arrested heart and strengthens and increases the frequency of a slowed and much weakened heart.

The table further shows that the antagonistic action of pilocarpine is displayed more obviously in increasing the strength than the frequency of the contractions.

Here we have another remarkable instance of an antagonistic action between two drugs otherwise so similar in their effect on the animal body: an instance, too, of antagonistic action in an organ though their effect when separately applied is similar; for both muscarin and pilocarpine slow and weaken or even arrest the heart, and yet, as we have just seen, pilocarpine antagonizes the action of muscarin.

It may possibly be said that the improvement from the application of the pilocarpine is a mere coincidence, and would occur irrespective of it; but to this we answer that in all our experiments prior to the application of the pilocarpine no improvement took place, whilst in every instance more or less amendment occurred immediately after the pilocarpine. Still we think it well to give experimental evidence on this point.

We therefore resolved to perform a set of comparative test experiments:

i. The action of muscarin on the heart.
ii. Of muscarin followed by pilocarpine.
iii. Of muscarin followed by atropia.
iv. Of muscarin followed by duboisia.

We destroyed the brains of three frogs and then laid bare their hearts. To the first we applied muscarin only. To the second we applied first muscarin, and when this had affected the heart we applied pilocarpine. To the third we applied first muscarin, and when the heart became fully affected we applied atropia. By means of these comparative experiments we can ascertain the degree of antagonism which pilocarpine and atropia manifest to muscarin, and we are able to compare the degree of antagonism exerted by pilocarpine with that exerted by atropia.

We used a 2 p. c. solution of nitrate of pilocarpine, a 1 in 120 solution of sulphate of atropia, and a solution of muscarin containing one part of alcoholic extract of amanita muscaria in four of water, and a 1 in 120 solution of the alkaloid of duboisia. The pilocarpine and extract of ammonita solution has a slight acid reaction. The results are given in Tables II. to V.

The experiments given in Table II. fully confirm the previous ones. They show that the heart does not recover after it is slowed or arrested by muscarin, and consequently that the improvement following the application of pilocarpine is not a natural recovery that would have occurred independently of the pilocarpine, but is due to the action of that drug. We now summarize the results given in Tables I. III. IV. and V.

In the first experiments given in Table I. extract of muscaria stopped the heart in four cases and almost stopped it in the remainder, whilst pilocarpine increased the pulsations on the average 18 beats, induced good cardiac contractions in seven cases, fairly good in one, and restored merely slight movement in the auricle in one case after arrest of the heart.

In the six experiments given in Table III. the muscaria stopped the heart in two cases and almost stopped it in the rest, whilst the pilocarpine increased the pulsations 16 beats on the average, and in three instances induced very good pulsations, in one good, and in two fairly good.

Our second series of experiments, Table III., confirms the first, Table I., in showing that, in a heart weakened and slowed by extract of muscaria, pilocarpine restores the strength even more than the frequency of the beats.

Our second series of observations enables us to compare the antagonistic action of pilocarpine with that of atropia and duboisia. The average increase in the number of beats after atropia and duboisia is as follows: after atropia 28, after duboisia 30.

Both in the experiments with atropia and duboisia the heart in every instance was stopped or almost stopped, and again on the application of either atropia or duboisia the drug in every case induced good pulsations.

The antagonism of pilocarpine for extract of amanita muscaria is not so marked as the antagonism of atropia or duboisia for muscarin. Atropia and duboisia increasing the pulsation of a heart stopped by muscarin on the average about 30 beats per minute, whilst pilocarpine augments the beat only to about 16 to 18 per minute; but the increase in the strength of the beat was as great after the application of pilocarpine as after the application of atropia or duboisia.

The greater antagonizing power of atropia and duboisia over pilocarpine is shown by the longer time the heart continues to beat after the application of atropia or duboisia, thus after

pilocarpine the heart stopped on the average in	28	hours
atropia	47	„
duboisia	49	„

We next adopted another method of comparing the antagonism of pilocarpine with the antagonism of atropia for muscarin.

We first arrested the heart with extract of amanita muscaria, and then allowed 8, 15, and 25 minutes to elapse before we applied either the pilocarpine or the atropia, using solutions of the same strength as in the previous experiments. The results we give in Tables VI. and VII.

Table II.

Action of muscarin on the Heart.

Jan. 10	The heart beating at	40,	fell in a	$\frac{1}{4}$ of an hour	to	4,	and stopped in	an hour	and did not begin again.
„ 14	„	44	„	5 minutes	„	5	„	25 minutes	„
„ 15	„	40	„	7 „	„	4	„	12 „	„
„ 21	„	36	„	9 „	„	4	„	28 „	„
„ 22	„	36	„	7 „	„	4	„	23 „	„
„ 24	„	36	„	8 „	„	12	„	20 „	„

Table III.

Antagonism of pilocarpine to muscarin.

Date.	After application of muscarin pulsations fell	After application of muscarin heart became	After application of pilocarpine pulsations rose	After application of pilocarpine heart became	Pulsation continued
Jan. 10	in 2 min. from 40 to 4	very feeble, all but stopped	in 17 min. to 20	very good	2 hours after became very weak; 12 hours after beat feebly at 4 per min.
„ 14	in 15 „ 40 to 4	„ „	in 17 min. to 10, and in 44 min. to 12	fairly good	stopped in 4 hours
„ 15	in 7 „ 40 to 4	„ „	in 6 min. to 10, and in 12 hours to 20	very good	15 hrs. after good at 20; 40 hrs. after at 16; stopped in 48 hrs.
„ 21	in 9 „ 40 to 2 stopped in 28 min.	stopped	in 9 min. from 0 to 20	good	36 hrs. after good at 20; 60 hrs. after good at 10; stopped 66 hrs.
„ 22	in 7 min. from 22 to 4 stopped in 13 min.	stopped	in 14 „ 0 to 20	very good	24 hrs. after good at 16; stopped 31 hrs. after
„ 24	in 2 min. from 38 to 4	almost stopped	in 2 „ 4 to 12 in 15 min. to 20	fair	stopped in 7 hrs.

Table IV.

Antagonism of atropia to muscarin.

Date.	After application of muscarin pulsation fell	Pulsations became	After application of atropia the pulsation rose	The pulsations became	Pulsations continued
Jan. 10	in 2 min. from 40 to 4	very feeble	in 2 min. from 4 to 32	very good	12 hours after pulsation very good at 30, and 84 hours after beat fairly well at 36
„ 14	in 13 „ 60 to 6	very feeble	in 1 „ 6 to 40 & in 30 min. rose to 52	„	36 hours after beat well at 36, and 48 hours after very feebly
„ 15	in 12 „ 52 to 0	stopped	in 1 min. 0 to 34	„	50 hours after beat well at 36
„ 21	in 4 „ 40 to 4 stopped in 28 min.	stopped	in 2 „ 0 to 20	good	10 hours after had stopped
„ 22	in 3 min. from 28 to 4	very feeble	in 3 „ 4 to 24	„	36 hours after beat was at 26; 44 hours afterwards stopped
„ 24	in 4 „ 28 to 2	almost stopped	in 1 „ 2 to 24	„	12 hours after good at 24

Table V.

Antagonism of duboisia to muscarin.

Jan. 14	in 5 min. from 48 to 0	arrested for 3 min.	in 3 min. from 0 to 40	very good	
„ 15	in 7 „ 52 to 0	stopped	in 3 „ 0 to 28	good	15 hours after beat fell at 24; stopped in 36 hours
„ 21	in 7 „ 40 to 4 in 24 stopped	stopped	in 3 „ 0 to 36	very good	36 hours after good at 24; 59 hours beat feebly at 16; stopped in 56 hours
„ 22	in 3 min. from 24 to 8 and in 27 min. to 4	very feeble	in 3 „ 4 to 20 and in 2 hrs. rose to 24	„	63 hours after good at 24
„ 24	in 9 min. from 36 to 4	almost stopped	in 1 min. rose to 30	good	12 hours after good at 28

Table VI.

Date.	After the application of extract of amanita muscaria pulsations stopped in	After the application of pilocarpine the pulsations begun in	and rose to	and became	Pulsations before application of muscarin.
An interval of eight minutes after arrest by muscarin.					
Feb. 26	29 min.	2 min.	24 in 44 min.	good at once	40.
An interval of fifteen minutes after arrest by muscarin.					
Feb. 26	65 min.	4 min.	20 in 20 min.	very good at once	38.
An interval of twenty-five minutes after arrest by muscarin.					
Feb. 27	at once	not at all			40. The heart was watched for 4 hrs.
Feb. 28	3 min.	,,			24 to 28.
Mar. 3	48 min.	34 min.*	20 in 34 min. to 32 in 55 m.	very good	56. 14 hours afterwards the ventricle beat poorly at 8.

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* The auricle begun to beat 8 minutes after the application of pilocarpine. In our figures in these tables we refer to the ventricle.

Table VII.

Date.	After the application of extract of amanita muscaria pulsations ceased in	After the application of atropia the pulsations begun in	and rose to	and became	Pulsations before application of muscarin.
An interval of eight minutes after arrest by muscarin.					
Feb. 26	20 min.	3 min.	32 in 75 min.	good at once	40.
An interval of fifteen minutes after arrest by muscarin.					
Feb. 26	29 min.	7 min.	12 to 20 in 7 min.	fairly good	40.
An interval of twenty-five minutes after arrest by muscarin.					
Feb. 27	at once	11 min.	20 in 19 min.	good	48.
Feb. 28	14 min.	10 min.	14 in 16 min.	good	56. 14 hours afterwards beat at 8, but had become weaker.
Mar. 3	17 min.	17 min.	28 in 100 min. 36 in 14 hrs.	very good	56.

Our experiments then show that pilocarpine, a sweater and salivator which slows and weakens or arrests the frog's heart, antagonizes, as regards the heart, the action of extract of amanita muscaria (muscarin), a substance which is also a sweater and salivator, and slows and also weakens or arrests the frog's heart.

In this respect pilocarpine is but little inferior to atropia and duboisia.

ON THE PHYSIOLOGICAL ACTION OF NARCISSIA, AN ALKALOID OBTAINED FROM THE BULB OF THE COMMON DAFFODIL (Narcissus Pseudo-Narcissus).

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In May 1877, Mr Gerrard, Teacher of Pharmacy at University College, London, extracted an alkaloid from the bulb of the common Daffodil and named it Narcissia. He placed in our hands a solution of the sulphate, and we now proceed to record the results of our investigations on the action of this alkaloid.

In Balfour's Botany, we learn that the daffodil possesses narcotic flowers and emetic bulbs. Our investigations show that narcissia is very closely allied to atropia; at least the narcissia obtained from the bulbs of the flowering plant, whilst strange to say the alkaloid obtained from the bulbs some time after flowering is over has a different action.

The alkaloid from the flowering plant dries the mouth, checks perspiration, probably checks the secretion of milk, widely dilates the pupil, greatly quickens the pulse, and antagonizes the effect of muscarin and pilocarpin on the heart. Narcissia is, however, a much weaker alkaloid than atropia, three-quarters to a grain of the former being about equivalent to $\frac{1}{60}$ to $\frac{1}{100}$ grain of the latter. Narcissia excites vomiting, but this occurred only after very large hypodermic doses and we have never seen emesis in man after the hypodermic injection of $\frac{3}{5}$ to $\frac{4}{5}$ grain. The alkaloid extracted from the bulbs after flowering time has the curious property of producing copious salivation, but in other respects corresponds to its fellow alkaloid. The differences which we can detect between the effects of the narcissia from the flowering plant and of atropia are first, a difference in their strength; second, narcissia induces sickness, whilst atropia does not; and third, that narcissia given internally contracts the pupil; and lastly, when applied locally, causes slight contraction before it produces dilatation.

We shall first speak of the alkaloid extracted from the bulb of the flowering plant.

I. The alkaloid obtained from flowering bulbs.

We first injected $\frac{1}{5}$ and $\frac{3}{5}$ of a grain respectively of the sulphate under the skin of the back of two frogs. The smaller dose produced no weakness, nor any effect beyond causing the animal after some hours to vomit a large number of worms. The larger dose produced some dullness but very little weakness, the animal remaining quiet till disturbed, and then moving fairly well. Respiration became slow and very shallow, indicated only by a slight movement under the chin. The animal remained in a dull state and died on the third day.

We next tested the effects on a moderate sized cat. We first injected at 7.30 A.M. one grain of the sulphate under the skin of the back. The animal in 10 minutes became dull, and its pupils dilated and were unaffected by light. Then followed very slight general weakness, and the pupils became more widely dilated. In 23 minutes the animal vomited, preceded for a few seconds by much convulsive retching. In an hour and ten minutes the animal begun to improve. In two hours and a half the pupils were decidedly less dilated; and in seven hours they were nearly natural, but the animal still remained quiet and indisposed to move.

Next day at 7.30 A.M. we injected $2\frac{2}{5}$ gr. under the skin. In 3 minutes the pupils were widely dilated. In 12 minutes it begun to retch, bringing up only a few drops of fluid after violent convulsive efforts. This dose produced decided weakness, but the animal throughout could jump off the table and run about the room; but when left alone it at once lay down on its side, its head lying on the ground. There was slight twitching of the lips and head, and the mouth was excessively dry. All the next day, the mouth continued dry and the pupils widely dilated.

After an interval of two days at 7 A.M. we injected a grain. This dose again widely dilated the pupils and made the mouth very dry but produced no sickness nor retching. Next morning the pupils were still dilated but the mouth was moist.

We next tried the effect on two men, dropping a little of a solution of 1 in 5 into one eye. In each case the pupil became widely dilated.

We had thus ascertained that narcissia dries the mouth, widely dilates the pupil, produces slight weakness and retching with vomiting.

The dryness of the mouth and the action on the pupil suggested the idea that narcissia might possess some of the other properties of atropia, and this clue we followed up in our subsequent investigation.

To some of our patients we carefully administered the sulphate of narcissia hypodermically and ascertained that two to three-fifths of a grain are required to cause slight dryness of the mouth, though with some persons much larger doses are required.

A young woman aged 18 was admitted with tape-worm and epileptoid fits. Her hands were always wet with perspiration, which could be seen as well as felt. We determined to test the power of narcissia to check this sweating. On June 6th we injected $\frac{3}{5}$ of a grain of sulphate of narcissia under the skin of the arm. In twelve minutes the hands were quite dry and so remained during the hour and three quarters we watched her. In twenty-five minutes she complained of dryness of the mouth. Her pupils became decidedly contracted in fifteen minutes and rather dilated in thirty minutes. Her pulse in half an hour rose to 120, i e. an increase of 36 beats. In an hour and forty minutes it had fallen 24 beats. In the evening she complained of frontal headache.

On June 8th we repeated the injection. In nine minutes her hands were drier, in twelve minutes quite dry, in forty-one minutes some moisture returned, in nine minutes the pupils became decidedly contracted and so remained. At first the pulse fell 10 beats in 6 minutes; in twelve minutes it rose 8 above the initial beat and in 23 minutes 22, beating 124 in the minute. After this the pulse gradually fell and in an hour after the injection it was 112. She never complained of any dryness of the mouth.

It thus appears that narcissia promptly checks sweating and in doses which produce very slight or scarcely any dryness of the mouth. In this respect again it acts like atropia. We found also that it greatly increased the pulse beat, in this respect too corresponding to the action of atropia.

In both these experiments the drug decidedly contracted the pupil, the contraction in one experiment giving way to slight dilatation. We noticed that at one time on looking at a strong light the pupil was decidedly more contracted than before the injection, whilst if the eye was shaded, the pupil was decidedly larger than before the injection. The pupil seemed in an unstable condition, oscillating a good deal on looking at a strong light. It would appear therefore that in moderate doses this alkaloid causes decided contraction of the pupil.

We next tested the antagonism between pilocarpine and narcissia in order to ascertain whether it could check the sweating and other symptoms produced by pilocarpine.

On June 2 we injected into a healthy person hypodermically half a grain of pilocarpine which almost immediately produced considerable perspiration and salivation. In eight minutes we injected $\frac{3}{5}$ gr. of sulphate of narcissia. In five minutes salivation greatly declined and the suprapubic pain produced by the pilocarpine had diminished. In 7 minutes salivation ceased; in 12 minutes, perspiration ceased, the skin becoming quite dry.

On June 12th we repeated the experiment on a patient with a very slight attack of gout in the left great toe. For this he had taken in the morning a drachm and a half of colchicum wine which, as on a former occasion, had quickened the pulse to 120 per minute and made it very weak and irregular. At two o'clock we injected hypodermically half a grain of pilocarpine.

In three minutes very profuse perspiration broke out over his whole body though previously his skin was quite dry, and he says he scarcely ever sweats. His hands and arms were drenched and large drops of sweat stood on his face. There was no salivation. The pilocarpine increased the pulse to 128 and 132, rendering it much more irregular and very much weaker. The patient was pale and felt faint. Eight minutes after giving the pilocarpine we injected $\frac{4}{5}$ gr. of nitrate of narcissia. In three minutes the perspiration became decidedly less and in 5 it had completely ceased; for on wiping his left hand arm and left side of face they remained quite dry.

In 7 minutes his pulse had fallen to 80; in 22 minutes it beat 88 and had become much stronger and much less irregular.

These experiments therefore show that like atropia, narcissia checks sweating and the salivary secretion.

We now proceed to record some further observations intended to elicit further particulars concerning the effect of narcissia on the pupil.

The topical application to an eye produced curious results. In a few minutes both pupils became contracted, the doctored eye in most cases being affected more than its fellow, which in some cases remained unaffected. In one case the contraction of the doctored eye was extreme, the pupil being reduced to one-eighth, having been previously a large one-third. This contraction was followed by dilatation of the narcissized eye, the opposite undoctored eye at the same time as usual growing still smaller. There was generally great dilatation, the pupil being increased to two-thirds or three-fourths, or even greater.

Table I. shows the results of our observations.

Table I.

Solution 1 *in* 10.

No. of Experiment.	Contraction begun.	Dilatation begun.	Dilatation lasted.	
1		20 min.	6 days	
2		20 min.	3 days	
3		35 min.	2 days	
4		25 min.	4 days	
5	5 min.	20 min.		Doctored eye only contracted
6	7 min.	26 min.	36 hours	Both contracted, doctored eye most
7	4 min.	25 min.	3 days	„ doctored eye earlier and most
8	4 min.	21 min.	3 days	„ doctored eye most
Solution 1 *in* 5.				
9	5 min.	20 min.	6 days	Both decided and equally contracted
10	5 min.	22 min.	9 days	Doctored eye only contracted; reduced from $\frac{1}{3}$ to $\frac{1}{8}$
11	3 min.	20 min.	more than 8 days	Both contracted, but doctored eye most
12	3 min.	20 min.	more than 5 days	Both contracted, doctored eye most
Solution 1 *in* 3 (same prep. as used in 1 in 5).				
1	No dilatation			Both contracted, doctored eye most
2	No effect			
3	Very slight dilatation			
4	No effect			
5	Very slight dilatation			

In the earlier experiments, though we noticed the fact of contraction, we failed to record the exact time of its onset.

It will be seen from this table that the primary contraction began usually in about five minutes and lasted ten to fifteen minutes. Usually the contraction, though distinct, was slight, and as we have said affected both eyes, though most the doctored eye; in some cases indeed the contraction was much greater in this eye. The contraction when it gave way was followed by very rapid dilatation, especially after the stronger solution. Thus in five to seven minutes in some cases it dilated from a small quarter to three-quarters. With respect to rapidity in effecting dilatation we suggest that it exceeds atropia.

The dilatation lasts a variable time. The 1 in 10 solution induced a dilatation lasting on an average nearly three days.

With a stronger solution, 1 in 5, the dilatation persisted longer, on an average in our cases of seven days.

We are led to conclude that narcissia paralyses accommodation less than atropia. At least our patients complained of very little dimness or haziness, even when the pupil was widely dilated; but we must admit that our observations on this point are not sufficiently accurate to justify a positive conclusion.

In Table II. we give the effect of the hypodermic injection of narcissia on the pulse.

Table II.

Sex.	Age.	Dose injected.	Pulse before injection.	Pulse rose to	Continued quick	Condition of patient.
F	19	$\frac{3}{5}$ gr.	84	128	2 hours	Epileptic
,,	,,	$\frac{3}{5}$ gr.	92	124		,,
M	39	$\frac{4}{5}$ gr.	64	116	5 hours	Convalescent from Delirium Tremens
M	62	$\frac{4}{5}$ gr.	No effect on pulse			Jaundiced*
,,	,,	$1\frac{1}{2}$ gr.	No effect on pulse			Jaundiced*

These observations, though few, show that narcissia quickens the pulse.

The similarity between many of the effects of narcissia and of atropia led us next to experiment on the heart, regarding the antagonism between narcissia and muscarin respectively, and between narcissia and pilocarpine. We shall first speak of the antagonism between narcissia and muscarin.

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* The only effect in these cases was slight but distinct contraction of the pupil. As this man appeared to resist the action of narcissia, we tested the effects of $\frac{1}{60}$ gr. atropia given hypodermically. This also produced no effect except to quicken his pulse 10 beats, but neither drying his mouth nor dilating his pupil.

We divided the cord of a frog opposite the occipito-atlantoid membrane, and thrust a wooden peg into the cavity of the skull to destroy the brain. When the effects of shock had passed away we opened the thorax and laying bare the heart found it beating 40 in the minute. We next made a small opening into the pericardium, and poured into it a small quantity of a solution of muscarin (1 in 4). Twenty minutes afterwards the beats fell to 22, whilst the contractions of the cavities became much feebler, and the auricular and ventricular movement lost its almost continuous action, a distinct though slight interval elapsing between the contraction of the auricle and that of the ventricle. We next poured into the pericardium a small quantity of a solution of sulphate of narcissia (1 in 10). In two minutes the beats per minute rose to 26, but in nine minutes they fell to 14, and remained between 12 and 14 during the next hour and a half. Though the narcissia failed to quicken the heart's contractions, in other respects it greatly improved them; thus previous to the injection of the narcissia there was very slight ventricular contraction, the cavity being widely dilated; but after the narcissia the contraction of both auricles and of the ventricle became very much stronger and more complete, and the ventricular contraction immediately followed the auricular.

Judging from this experiment, narcissia would seem to obviate some of the effects of muscarin, strengthening the auricle and ventricle, and removing the interval between the auricular and ventricular contractions.

We repeated this experiment, applying to the exposed heart a small quantity of muscarin solution (1 in 4), but without any effect either on the frequency or force of the heart's contractions. After waiting half an hour we applied a little of the nitrate of pilocarpine solution (1 in 12), and in five minutes the beats fell from 36 to 22 per minute and continued at this rate, though the strength of the contraction grew weaker and weaker, so that in 16 minutes the heart beat very feebly. We then applied to the heart a little of the sulphate of narcissia solution (1 in 5). The first effect lasting about a minute was a decided increase in the cardiac weakness; indeed the heart all but stopped. In two minutes, however, the contraction became far stronger than previous to the medication; in fact the ventricular contraction was fairly strong, the walls of the cavity becoming firm and pale. The heart beat 22 per minute. In four minutes the ventricle, still at 22 per minute, beat very strongly. An hour and a half after the application of the narcissia, and two and a half hours from the commencement of the experiment, the ventricle stopped in wide diastole, and then only an occasional auricular contraction occurred.

In some subsequent experiments we found that on the application of the muscarin solution the heart was often not at all or scarcely affected either in frequency or in force, so that we were unable to test the antagonistic action of narcissia. We therefore suspended this investigation to test the antagonism between narcissia and pilocarpine. Our muscarin was synthetically prepared by Merck of Darmstadt, and our experiments lead us to conclude that this preparation or the specimen sent us exerts a far feebler action on the heart than the muscarin extracted from the fungus.

We again tested the antagonism between pilocarpine and narcissia. We prepared a frog as in the previous experiments, and having laid bare the heart found it beating 38 per minute. We then poured into the pericardial sac a little solution of nitrate of pilocarpine (1 in 12). The heart immediately stopped in diastole, all the cavities being widely distended with blood; and during the next three minutes the heart remained in this condition, mechanical stimulation meanwhile failing to excite contraction. We then poured into the pericardium a very small quantity of the sulphate of narcissia solution, and at once the heart recommenced beating vigorously 18 per minute, and in twenty-two minutes it beat strongly 24 per minute, and during the hour we watched this organ it never beat any quicker. In six hours we found the heart arrested in wide diastole. Here the effects of the narcissia were very decided. It obviated the inhibitory effects of the pilocarpine, and restored strong vigorous contraction to the arrested heart.

We repeated this experiment on another animal, applying to the exposed heart a little of the nitrate of pilocarpine solution (1 in 12). In one minute the heart beat much feebler, but its frequency remained much the same. In six minutes the beats had fallen from 39 to 14, and the contraction was so feeble that the heart was brought nearly to a standstill, only the slightest movement being visible in the ventricle. The diastolic pauses were of very irregular length.

We then applied a little of the narcissia solution, and the heart immediately ceased beating, all its cavities being widely distended with blood. In two minutes very slight contraction commenced in the auricles: this contraction increased, and in four and a half minutes the ventricle began to contract, at first very feebly, at the time the auricles were contracting well. In five minutes the ventricle too was contracting well. In seven minutes the cavities contracted 21 times per minute as strongly as in health. In twelve minutes the pulsation had fallen to 16.

It thus appears that narcissia corresponds to atropia in its antagonistic action to muscarin and pilocarpine on the heart. With us muscarin manifested far less effect on the heart than pilocarpine; but in the experiment in which muscarin slowed and weakened the heart, narcissia in a great measure obviated these effects, but it increased the strength rather than the number of the beats.

The antagonism between narcissia and pilocarpine is much more marked. Pilocarpine had a quick and very decided effect on the heart, and in one experiment it had nearly arrested the action of the heart, though it still beat 20 per minute. Narcissia at first completely stopped the heart, then the auricle begun to beat a little, and next the ventricle, and soon both cavities were beating with normal strength but still with much diminished frequency. Both with muscarin and pilocarpine the narcissia improved the strength of the beat more than restored the lost frequency. If pilocarpine slows and weakens the heart, through the inhibitory action of the vagus, then narcissia seems to obviate the inhibitory action which weakens but not the inhibitory action which slows the heart. It is usually held that pilocarpine stimulates the terminations of the vagus, and perhaps the intra-cardiac inhibitory ganglia, and thus slows and weakens the heart, and that atropia paralysing these structures obviates the effects of the pilocarpine. The same explanation may be offered of the action of narcissia, though if the foregoing hypothesis as to the action of pilocarpine be correct, it is singular that narcissia, by paralysing the vagus and the intra-cardiac inhibitory ganglia, should not remove the slowing as well as the weakening effect produced by the stimulation of the inhibitory apparatus.

Action of Narcissia on the Frog's Heart. We have already learned that like atropia, narcissia antagonizes the action of muscarin and pilocarpine on the frog's heart. Now we will endeavour to ascertain the effect of narcissia itself on the heart.

We proceeded in the following way: we divided the cord opposite the occipito-atlantoid membrane and thrust a small peg of wood into the skull to destroy the brain and arrest hæmorrhage. Then we opened the chest and counted the heart's beats. During the first ten minutes the beats fell from 52 to 34 and so remained during the next 8 minutes. We then partially opened the pericardium and poured into the sac a small quantity of the sulphate of narcissia solution (1 in 5). In 14 minutes the beats fell from 36 to 26 and the action of the heart was slightly modified, the ventricle during the greater part of the diastole becoming flaccid and distended with blood, though still the cavities beat vigorously. Two hours from the application of the narcissia the beats had risen to 38 and in 3 hours to 44 per minute. Next morning the heart was beating vigorously at 39 per minute and we again applied narcissia solution. In 5 minutes the beats had fallen to 25 and 24 per minute and were more feeble, the auricular and ventricular action being less continuous. The beats continued 4 hours at this rate: in 5 hours the heart stopped in wide diastole.

This experiment we repeated, but on this occasion we allowed a much longer interval to elapse before applying the narcissia, to allow ample time for the effect of shock to pass away. Thus we opened the thorax at 7.40 A.M. but delayed the application of the narcissia till 1.39, at which time the heart beat 42 per minute. In 5 minutes the narcissia reduced the beats to 22 and so continued about an hour, when the beats became faster and rose in 3 hours to 38; whilst slowly beating the contraction seemed but little weakened, and this weakness passed away as frequency returned.

In another experiment, after laying bare the heart, we allowed 17 hours to elapse before applying narcissia. Just before this the heart beat 44 per minute, but in 11 minutes after applying the drug, the contractions fell to 24 with a rather weaker beat, but after this they begun to rise and in 50 minutes the heart beat 34.

In many of our experiments the heart was strongly affected by the shock from dividing the sternum and pulling the parts aside and also from the occasional free bleeding this operation entailed. In the succeeding experiments we operated differently. After destroying the brain we merely opened the abdomen and incised the pericardium. The heart's action was but little affected. After two observations, and when we had ascertained that the heart beat at its normal rate, namely 46 to 48 per minute, we applied the solution of narcissia. In 3 minutes the beats fell to 24, and the heart certainly was not weakened. The diastole was very short and the systole strong and prolonged; in this respect differing from our previous experiments. In 2 hours the heart had recovered its normal frequency.

We throw all our observations into Table III.

Table III.

Date.	Heart slowed.	Maximum slowness reached in	Heart recovered.	
May 31	10 beats	14 min.	120 min.	During the slowing the heart beat a little feebly in these four experiments
June 1	15 beats	5 min.		
,, 1	20 beats	5 min.	180 min.	
,, 2	20 beats	11 min.		
Sternum not divided.				
June 2	24 beats	3 min.	120 min.	
June 4	26 beats	3 min.	120 min.	Heart weakened
June 5	14 beats	7 min.	Never recovered	
June 5	20 beats	3 min.	,,	
June 5	14 beats	6 min.	,,	
June 5	26 beats	3 min.	,,	
Alkaloid 3 (1 *in* 3 *solution*).				
July 17	35 beats	4 min.		
July 17	28 beats	3 min.		
July 17	44 beats	7 min.	Stopped altogether in wide diastole	
	46 beats	3 min.		

In all these experiments the heart stopped beating much sooner than in undrugged hearts. In the foregoing experiments we confined our attention to the frequency of the heart's action. We shall have another opportunity to speak of its influence on the strength and character of the contraction.

Is this slowing due to the narcissia or the water holding it in solution? To solve this question we applied water only to the heart and watched the effect. In the first experiment in dividing the sternum much blood was lost, so that the heart became small and whilst contracting propelled but little blood. In the other experiments we merely opened the abdomen and in this way reached and divided the pericardium. In the three experiments we made, the heart's contractions, both in respect to strength and frequency, were unaffected.

We therefore conclude that the slowing effect is due simply to the narcissia; and we draw attention to the curious fact that whilst narcissia antagonizes pilocarpine which slows and weakens the heart, narcissia itself also slows the heart.

As we found that narcissia applied directly to the heart, in situ, slowed its beats, and as there is so close a similarity between narcissia and atropia, we thought that atropia might act in the same way. We therefore tested the action of atropia on the heart and embody our results in Table IV. In these experiments, after pithing and pegging the animal, we opened the abdomen and then the pericardium, but without dividing the sternum. We used a solution of atropia 1 in 20.

Table IV.

Date.	Heart slowed.	Maximum slowness reached.	Heart recovered.	
June 3*	10 beats	6 min.	180 min.	
June 4*				Fell 10 beats in three minutes and then gradually and continuously fell 22 beats two hours and stopped at three hours. Heart ceased in wide diastole
June 5	30 beats	2 min.	120 min.	
June 5				A gradual and continuous fall from 48 to 12, when it soon ceased
June 9	4 beats	4 min.	30 min.	
June 9*a*	18 beats	5 min.		
June 9	11 beats	3 min.	120 min.	
June 9*a*	27 beats	1 min.		
June 9	14 beats	8 min.	70 min.	
June 9*a*	23 beats	7 min.		

In the experiments marked * the heart did not expand naturally during diastole and received but little blood. In the experiments marked *a* we used the same frog as in the immediately preceding experiment: thus we applied atropia between 7 and 8 in the morning, and at 6 that evening, when the heart had completely recovered and was beating vigorously, we a second time applied atropia.

Thus we find that atropia topically applied, slows the heart, thus affording another instance of the similar action of these two drugs.

It is singular that these drugs should themselves be able to slow and after a short time to arrest the frog's heart, and yet both will obviate the slowing and weakening effect of jaborandi and muscarin ; whilst neither is able to obviate the slowing induced reciprocally of each other.

We have thus ascertained that this alkaloid dries the mouth; checks perspiration; dilates the pupil; quickens the pulse, and antagonizes the action of muscarin and pilocarpine on the heart, skin, salivary glands, &c.; in all these respects corresponding to atropia.

II. The alkaloid obtained from bulbs which had flowered.

At this stage of our investigations the alkaloid was exhausted, and Mr Gerrard prepared another specimen for us. He used another supply of fresh bulbs, but the time for flowering had passed away several weeks. He prepared for us an alkaloid by the same process as the previous alkaloid, but with regard to them we thought it well to test the new alkaloid on a cat, not for a moment expecting that its effects would differ from those of the previous alkaloid. To our astonishment, instead of drying the mouth, dilating the pupil, and producing sickness, it caused copious salivation, and failed to affect the pupils. The salivation was profuse, the saliva for some time trickling in a rapid succession of drops. We give a short account of the experiment.

We injected under the skin of a small-sized cat 3½ grains of the alkaloid. For the first twenty-six minutes the cat remained unaffected; then we noticed profuse salivation. In thirty-six minutes some muscular weakness set in, with twitchings amounting almost to convulsions. Though weak the animal could easily walk, and retained considerable strength; indeed it seemed awkward rather than weak. In forty-two minutes the cat passed water freely, and the breathing became greatly hurried. In forty-five minutes the animal appeared to be delirious, and the strong muscular twitchings continued. In sixty minutes we counted the respirations and found them 118 per minute. In an hour and a half the salivation had greatly decreased. In three hours and a half the animal had considerably improved, the twitchings having ceased, and it seemed much less delirious. The mouth still ran slightly; throughout the experiment, there was no purging nor sickness, and the pupils were not dilated.

How was it that this alkaloid of daffodil differed so strikingly in its effects from the previous specimen? Was it due to our using a larger dose (3½ grains) of the new alkaloid? Or does the daffodil during and after flowering yield respectively a different alkaloid? We know of no instance of a plant yielding a different or modified alkaloid at different times of the year, so we were not at all inclined to accept this explanation.

Was the difference in the effects due to the largeness of the dose? This seemed improbable, since of the first prepared alkaloid, in the first instance we used only one grain, and on a second occasion $2\frac{2}{5}$th grains, simply with the effect of intensifying, not modifying, the symptoms with the stronger dose. However, we determined to settle this point by experiment.

Next day (July 22) the cat had quite recovered, and we injected into the connective tissue of the shoulder one-third grain of the second alkaloid (nitrate), and in two minutes the saliva began to run from the mouth.

Next day (July 23) the cat had quite recovered, but on moving her about salivation at once recommenced but soon ceased. When it had quite stopped we injected one-third grain of the second alkaloid into the loose connective tissue of the shoulder. In four minutes the mouth again began to drip, followed in five minutes with profuse salivation, long ropes of thick saliva reaching from its lips to the ground, and along these ran frequent drops of more watery saliva. The pupils were unaffected. We then injected $\frac{1}{40}$th grain of atropia solution (1 in 120), which in five minutes widely dilated the pupils, quite dried the mouth, and the animal violently vomited.

Next morning (July 24) we found the cat pretty well though rather quiet; the pupils still dilated but much less so than on the previous day; they acted pretty well to light, though the mouth was quite dry. Yet to our surprise, as soon as we moved the animal, profuse salivation commenced. Here we had profuse salivation, which after a time ceased, so long as the animal remained quiet; but even slight muscular exertion twenty-four hours afterwards brought on again profuse salivation. This experiment shows that atropia checks this salivation, but that the effects of the narcissia are more enduring.

It is obvious, therefore, that the difference in the dose cannot explain the singular difference in the action of the two narcissia alkaloids.

Action on Man. We next tested the effect of the alkaloid, administered by the mouth, on man.

We gave without effect to a man (H——), aged 40, suffering from renal calculus, half a grain of the nitrate. Next day we gave three-quarters of a grain. The pupils became, perhaps, a little dilated, but we were doubtful whether this was due to the drug or to the varying light. An hour later he felt a little sick, with sinking sensation at the epigastrium. Next day we gave a grain, which induced only slight faintness, coming on in a quarter of an hour, with slight drowsiness. In an hour he felt rather sick. On the following day (July 22) we gave this man a grain and half by mouth, and this dose induced slight though decided effects; it induced distinct salivation, beginning in ten minutes, but more marked an hour after the medicine; it caused slight running at the eyes; some sinking at the epigastrium, with slight nausea and hiccough, and it slightly contracted the pupils. The pulse was unaffected. Some hours afterwards he had a semi-solid motion. Next day (July 23) we gave him two grains, by mouth, with similar but still more decided effects. In eight minutes free salivation set in, with a sinking sick sensation, hiccough and heart-burn. His pulse was increased from 10 to 13 beats per minute. He spat about an ounce of saliva. Some hours after he passed a relaxed stool, though previously he had suffered from constipation. In none of these experiments did we notice any perspiration.

On July 25 we gave him two grains and a half. In seventeen minutes free salivation occurred, and in the course of the following half-hour he spat an ounce and a half of very ropy saliva. He suffered from slight hiccough, sinking, and a "faint feeling". His pupils became slightly contracted, and his pulse accelerated by 10 to 12 beats per minute. He did not perspire.

On the 26th we gave him two grains and three-quarters, which produced the same symptoms, salivation to the extent of an ounce of thick ropy saliva, quickening of the pulse 12 beats per minute, and the "sinking feeling".

On the 27th we gave three grains with the usual symptoms. Thus he spat just an ounce of saliva, experienced the usual sinking sensation at the epigastrium, his pupils became slightly contracted, but in this instance there was no quickening of the pulse, and about two hours after the alkaloid he passed a loose stool.

On the 28th he took four grains, followed by symptoms in some respects more marked. Thus he complained of greater faintness; he spat an ounce and a half of saliva; his pulse was not quickened nor his pupil contracted; and an hour and a half after the administration of the drug he voided two stools.

Next we experimented in a different way on the same man. We first gave him half a grain every two hours. This caused slight increase of saliva, without spitting, which passed off after the fourth dose, "rumbling in the stomach", but no nausea. We continued the drug for two days, and on the third day he took a grain every two hours. The second dose caused diarrhœa (five motions) in the day, with severe aching pain below the left false ribs. He felt ill. It produced no salivation nor nausea, no action on the skin, nor any alteration in the pulse.

To sum up. In man it produces free salivation of ropy saliva, slight contraction of the pupils, faintness, a sinking sensation, nausea, running at the eyes, hiccough, heart-burn, and slight relaxation of the bowels, and sometimes slight quickening of the pulse.

In our experiments on man in no instance did we produce sweating. Though it seemed highly improbable that a salivator would check sweating, yet we thought it advisable to determine this point by experiment. We therefore administered to a woman half a grain of pilocarpine twice, with an interval of thirteen minutes, and when wet with perspiration, and when the saliva had begun to be secreted abundantly, we injected hypodermically two-thirds of a grain of nitrate of narcissia. This we think increased both the salivation and sweating, certainly it did not in any degree check it, for the perspiration lasted eight hours.

We next tested the topical effect of the second alkaloid on the eye, and found that, like the alkaloid prepared in May, it dilated the pupil, though in a much less degree, both in amount and duration.

We give the results of our investigations in Table V.

It was evident that the effect of the second alkaloid prepared for us by Mr Gerrard differs curiously from the effect of the first. The first has most of the properties of atropia, but also causes vomiting.

The second alkaloid excites free salivation, and if it does not cause perspiration it certainly does not stop it. It purges somewhat. Internally administered it slightly contracts the pupils; locally applied it first contracts the pupil and then slightly dilates it, but much less than the first alkaloid.

Table V.

2nd Alkaloid. 1 *in* 3.

	Dilatation begun.	Amount of dilatation.	Dilatation lasted.	Primary action.
July 27	No dilatation			Both pupils rather contracted, the doctored eye the most
July 28	3 hours	Very decided	Few hours	” ” ”
1 *in* 5.				
July 31	125 min.	Very slight	Few hours	Both pupils contracted, the doctored eye much the most; reduced from $\frac{1}{3}$ to $\frac{1}{5}$
July 31	No dilatation			Slight primary contraction of doctored eye
Aug. 3	3 hours	Very decided, double other	9 to 10 hours	
Aug. 7	3 hours	Decided		Both pupils contracted, especially the doctored eye
Aug. 7	4 hours	Slight		Primary contraction of the doctored eye
1 *in* 10.				
July 19	No dilatation			Doctored eye contracted decidedly
July 20	No dilatation			” ” ”
1 *in* 5 *continued.*				
Aug. 7	3 hours	Decided	4 hours	Primary contraction of doctored eye
Aug. 7		Decided		” ” ”

3rd Alkaloid extracted with Chloroform. 1 *in* 5.

Aug. 10. Very slight dilatation

III. The extract of flowered bulbs.

Hitherto we had used the alkaloid. We now tried the extract, and Mr Gerrard prepared us a specimen from the roots after flowering had ceased. We first tested the effect on man and afterwards on a cat.

To the man on whom we made many observations with the alkaloid, we gave three drops of a solution of the extract (1 in $1\frac{1}{2}$), which produced slight but decided salivation, without any other effect.

Next day (July 30) we gave him six minims of the mixture (1 in $1\frac{1}{2}$). This moistened his mouth a little, and produced slight feeling of sinking, and in two hours and a half he passed a watery stool, preceded for an hour and a half by "rumblings in the bowels." There was some salivation.

On July 31 we gave him twelve minims of the mixture (1 in $1\frac{1}{2}$), and in five minutes he became salivated, the salivation lasting about half an hour, and he spat half an ounce; in twenty minutes he felt very sick, the sickness lasting an hour. In an hour he had "rumbling in his stomach;" in an hour and twenty minutes he passed a watery motion without griping; and in two hours another watery and much more copious stool; a third, also watery, in about four hours. His pulse in twenty minutes increased 12 beats per minute.

We next tested the extract on a lad, aged 15, completely recovered from acute Bright's disease.

On July 31 he took two five-minim doses of the 1 in $1\frac{1}{2}$ solution, at three hours interval, without any effect. On the following day we gave him ten minims; in half an hour he vomited, and again in an hour and a half. In other respects he was unaffected.

On July 31 Mr D. F. Dymott, a gentleman at that time acting as my clinical clerk, took twelve minims of the mixture. In four minutes his mouth became moister, and so continued fifteen minutes; in twenty minutes he felt sick; in thirty-five minutes he vomited, with a slight amount of retching; and again in forty minutes. His pulse increased twelve beats, possibly from the nausea.

These experiments show that the extract acts as an emetic and purgative, its emetic being more marked than its purgative action. It also slightly increases the flow of saliva, and quickens the pulse a little, possibly in some cases from the nausea.

We conclude that the daffodil bulb contains two active principles—the alkaloid and another unknown substance. This conclusion is based on the following facts. Mr Gerrard calculates that a grain of the alkaloid is contained in about nineteen minims of the extract. We gave to H———, on whom we experimented with the extract, one, two, and three and four grains of the alkaloid. Four grains (equal to 114 of our extract mixture) produced salivation ($1\frac{1}{2}$ oz.), slight faintness, and two watery stools; whilst twelve minims of the extract solution, equal to eight grains of the extract, and less than one-half a grain of the alkaloid, produced much less salivation, but made him feel sick and produced three large watery motions. The same dose on Mr Dymott produced a doubtful trace of salivation, no diarrhœa, but excited vomiting twice. We therefore think the alkaloid is the salivating agent, but some other constituent excites the nausea, vomiting and diarrhœa.

Action of Extract on Cat. We injected fifteen drops of a solution of the extract (1 in $1\frac{1}{2}$) under the skin just behind the shoulder. Almost immediately slight salivation set in, and the eyes watered, and the nose became wet. These effects continued and decidedly increased during the hour we watched the cat. The salivation at its height was very profuse, the saliva dripping freely from the mouth and strings of ropy saliva hanging from the jaws. In twenty minutes the animal became decidedly weakened, walked and ran rather unsteadily, and apparently used its hind less perfectly than its front legs; when left alone it lay down and placed its head on the floor. There was much muscular twitching and tremor. In thirty-five minutes it begun to regain its lost power. In fifty minutes it was violently sick; the pupils were unaffected. At the end of an hour when we left it it was still weak and tremulous. We returned in half an hour, but the cat had escaped, having been again sick during our absence.

IV. The extract of flowering bulbs.

As our results were so unexpected, being ignorant of any other instance of a plant having some of its properties completely reversed at different seasons of the year, we thought it better to postpone the publication of these investigations, and to re-test in the following spring the action of an extract prepared from the bulb of the flowering plant. In April of this year (1878) Mr Gerrard kindly prepared an extract, and on the 11th we injected one and a half grain under the skin of a cat's back. In twenty-three minutes the cat vomited food; in twenty-six minutes it repeated the vomiting; in twenty-nine minutes it passed a rather slimy motion; in thirty-one minutes the mouth became a little dry; in sixty-two minutes the cat again retched violently; in sixty-seven minutes we injected another grain and a half of the extract; in sixty-nine minutes the cat again defæcated; its mouth was decidedly dry; in seventy-four minutes the animal was dull and sleepy; in eighty-four minutes it again vomited, the mouth continued dry but not extremely so; in 111 minutes it vomited again; in 114 minutes it passed a slimy motion; in 142 minutes we injected two grains of extract; and in 182 minutes $3\frac{1}{2}$ grains; soon afterwards the cat became very sleepy and its mouth decidedly drier. Next morning, seventeen hours after the experiment, the cat seemed very heavy and sleepy, with its mouth very dry, much drier indeed than during the experiment on the previous day. The pupils were never dilated, and the animal seemed only a little weak. This experiment with the extract confirms for the most part the results obtained with the alkaloid from the flowering plant the previous year, for it produced a great dryness of the mouth.

SUMMARY.

Our investigations we venture to believe show that the bulb of the flowering plant

I. Dries the mouth.

II. Checks the cutaneous secretion.

III. Dilates the pupil, especially on topical application to the eye, the dilatation being preceded for a short time by contraction.

IV. Quickens the pulse.

V. In a great measure it antagonizes the effect of muscarin and pilocarpine on the heart of frogs.

VI. Directly applied to the frog's heart it slows and weakens its contractions.

VII. Causes retching and vomiting.

VIII. Slight general weakness, and

IX. Drowsiness.

And our investigations further show that the alkaloid extracted from the bulb later in the year when flowering is over

I. Causes copious salivation.

II. Probably increases cutaneous secretion.

III. Internally applied it slightly contracts the pupil. Topically applied it dilates the pupil, but less so than the alkaloid of the flowering plant.

IV. It slightly relaxes the bowels.

V. Causes slight faintness, and

VI. Nausea.

We find that the extract, firstly, causes slight salivation, secondly, purges and vomits. The emetic and purgative effect is we think due not to the alkaloid, but to some other constituent.

ON THE PHYSIOLOGICAL ACTION OF THE COMMON SNOW-DROP (Galanthus Nivalis), AND OF THE JONQUIL.

By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London*, and E. A. MORSHEAD, M.R.C.S., L.R.C.P.

After the results described in the foregoing paper we determined to test the action of another amaryllidaceous plant, and chose the common snow-drop.

We first employed an alkaloid prepared for us by Mr Gerrard from the bulbs gathered four months after flowering time.

We injected nearly three grains of this alkaloid (14 minims of a 1 in 5 solution) under the skin of a cat. In three minutes lachrymation and salivation set in, and in eleven minutes became very free; in thirteen minutes the cat passed a very large quantity of water, and this was repeated six times in the following fourteen minutes, a very large quantity being voided each time. The lachrymation and salivation ceased in fifty minutes. Twenty-four minutes after the injection the cat passed a semi-solid motion. There was no vomiting, and scarcely any weakness.

To a lad aged 16, the subject of hæmophilia, but who at the time was otherwise in good health, I gave two grains of the nitrate of snow-drop alkaloid. It caused nausea in a quarter of an hour and much sickness in twenty-five minutes; and the sickness was repeated three times in an hour. His mouth was a little moister than before the experiment, but not more so than the nausea would account for. The pulse increased 12 beats; the pupil was not altered. In the afternoon he had three loose motions.

Next we tested the effect of the topical application of the alkaloid to the eye on two persons, dropping a little of a 1 in 5 solution into one eye of each. The alkaloid acted in all respects like narcissia. In one experiment both pupils became slightly contracted. In about an hour the doctored pupil began to dilate, and soon became considerably enlarged, and did not return to the size of the opposite pupil till the third day. In the other experiment both pupils in three minutes became contracted, and so continued for thirteen minutes, the pupils having contracted from one third to one-fifth; then the doctored pupil became dilated and so remained twenty-four hours.

These experiments then shew that the alkaloid extracted from the bulb after the plant has ceased flowering acts in a manner correspondent to that of the alkaloid of daffodil obtained from bulbs after the flowering time. We determined to wait till the following year, and to test the action of the bulb whilst the plant is in flower. Mr Gerrard kindly made us a little extract this year (1878).

On March 5 we injected eleven drops of a 1 in 2 solution under the skin of a middle-sized cat. In 24 minutes it vomited; in 28 it retched; in 33 minutes the breathing became much hurried and panting, 270 per minute: in 38 minutes the pupils were decidedly dilated. The animal then became weak, the mucous membrane of the mouth very pale, soon becoming rather livid. It then became convulsed, and died 43 minutes after the poisoning.

The effects of the alkaloid, then, are vomiting, dilatation of the pupil, but certainly no salivation. Judging from the extreme pallor of the mucous membrane it appears to be a powerful heart depressor.

On April 2 we injected six minims of a 1 in 3 solution of the extract under the skin of a small cat. In 19 minutes it vomited a small quantity of what looked like saliva; in 21 minutes the pupils were decidedly dilated, the mouth pale, and the breathing hurried; in 27 minutes the breathing became still more rapid, and the animal was convulsed, the mucous membrane of the mouth was pale and livid; in 31 minutes the animal died. In this experiment the effects were vomiting, dilatation of the pupil, probably cardiac weakness.

We determined to use a still smaller quantity, as the animals died too soon to enable us to investigate the effects of the drug.

On April 2 we injected into a full-sized cat two minims of a 1 in 3 solution of the extract. In 15 minutes the pupils became rather dilated and the mouth rather dry; in 24 minutes the pupils were still more dilated and mouth drier; in 32 minutes the cat vomited violently, with very little result; in 38 minutes the pupils were widely dilated and the mouth still drier, but not so dry as after a full dose of atropia; in 50 minutes the bowels acted, and the cat passed water freely, the pupils still dilated and the mouth dry; in 57 minutes the bowels acted again; in 68 minutes vomiting was repeated; in 70 minutes the mouth was moister. On the following day we injected into the same cat three minims of the 1 in 3 solution, which in 15 minutes widely dilated the pupils and made the mouth a little dry; in 20 minutes the cat vomited; in 30 minutes it again vomited, and the pupils were very widely dilated and the mouth dry; and in 35 minutes it vomited again, and the pupils were still more dilated; in 38 minutes the breathing became rather laboured; in 44 minutes the animal was weak and tumbled over on its side; the pupils were widely dilated and the mouth very dry. It passed water very freely. In 44 minutes it became too weak to stand; in 45 minutes it was convulsed, the mucous membrane of the mouth being very pale; and in 47 minutes it died. We immediately opened the chest and found the heart stopped and flaccid not distended. The strongest current from a one-celled du Bois-Reymond induction coil failed to excite any contraction, though we applied it to various parts of the heart. The other muscles of the body contracted readily under a weak current, and galvanic stimulation to the nerves caused the muscles to contract.

These two experiments confirm the foregoing, and show that the flowering bulb of snow-drop dilates the pupil, dries the mouth, causes vomiting, weakens and arrests the heart.

These experiments therefore show that galanthia differs like narcissia, according to the time of year the extract is prepared; that the alkaloid of the bulb from the flowering plant dries the mouth, whilst that from the plant after flowering has ceased acts in the opposite way and causes profuse salivation.

Physiological action of the Jonquil.

We then tested the action of an alkaloid extracted by Mr Gerrard from the bulb of the jonquil, after the plant had ceased flowering.

On July 5th we injected under the skin of a large cat two grains and three-quarters of the alkaloid.

It excited a flow of tears and saliva, beginning in thirteen minutes and continuing, though in a less degree, two hours and twenty minutes, when we discontinued our observations. The pupils were not dilated. The bowels were opened twice, the first motion being semi-solid, the second consisted of a little slime. The cat became a little heavy, but there was no appreciable muscular weakness. The alkaloid did not excite vomiting. This single experiment suggests that the alkaloid of the jonquil is similar in its action to those of the narcissus and the snow-drop.

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ON THE RELATIVE PARALYSING ACTION OF ATROPIA AND PILOCARPINE ON THE HEART.

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As is well known, the topical application of pilocarpine in large doses quickly slows and weakens the heart, but requires usually several hours to greatly weaken or arrest the heart. In our experiments we find that atropia also greatly slows and weakens the heart, and often after some hours arrests it; indeed it appeared to us that atropia affects the heart as powerfully as pilocarpine. We now record some experiments to test the relative action of atropia and pilocarpine.

We used a two per cent. solution of pilocarpine nitrate and atropia sulphate, and we experimented in the following way. We exposed the hearts of two brainless frogs, and then partially opened the pericardium and filled the pericardial sac of one with the pilocarpine, and of the other with the atropia solution.

We give our results in Table I.:—

Table I. Action of nitrate of pilocarpine topically applied to the frog's heart.

Date.	After application beats fell from	Beats became	Heart recovered in	Heart continued beating
May 29	42 to 24	weak		10 hours
,, 31	44 to 28	rather weak	36 good beats in 5 hours	36 hours
June 11	50 to 28	weak	did not recover	16 hours
,, 13	40 to 28	very weak	did not recover	9 hours
Average	27			17·7 hours

Action of sulphate of atropia topically applied to the frog's heart.

May 29	44 to 12	weak	3 hours	40 hours
,, 31	44 to 20	weak	5 hours	18 hours
,, 31	52 to 16	weak	30 feeble beats in 5 hours	16 hours
June 11	40 to 20	weak	did not recover	5 hours
,, 13	48 to 20	weak	did not recover	16 hours
Average	17·6			19 hours

These experiments show that atropia slows the heart even more than pilocarpine, for the average number of beats after pilocarpine was 27 per minute, after atropia 17·6 per minute.

When the maximum slowing is reached, we find that pilocarpine weakens the heart more than atropia.

We now give Table II., showing when the maximum slowing was reached :—

Table II. Maximum effects of pilocarpine.

Frequency.

May 29	in 2 minutes.
„ 31	in 3 minutes.
June 11	in 8 minutes.
„ 13	in 19 minutes.
	Average 8 minutes.

Strength.

May 29.	Grew progressively weaker till stopped.
„ 31.	In 15 to 20 minutes.
June 11.	Grew progressively weaker till stopped.
„ 13.	„ „

Maximum effects of atropia.

Frequency.

May 29	in 2 minutes.
„ 31	in 5 minutes.
June 11	in 30 minutes.
„ 13	in 3 minutes.
	Average 10 minutes.

Strength.

May 29.	In 2 minutes.
„ 31.	In 10 minutes.
June 11.	Grew progressively weaker till stopped.
„ 13.	„ „

We find that the topical application of atropia slows the heart more than the topical application of pilocarpine, but when the maximum slowness is reached the weakness is greater with pilocarpine than with atropia; and that the maximum slowing effects occur about the same time after the application of pilocarpine and atropia.

These experiments show that atropia does not simply paralyse the vagus, for paralysis of the vagus could not explain the slowing and weakening which follow the topical application of atropia. This must be due to the action of atropia either on the muscular substance of the heart or on the excito-motory apparatus. As atropia has no more action on voluntary muscle than common salt, it is improbable, we think, that the effects of atropia are due to its action on the cardiac muscle; and as atropia has a powerful action on the spinal cord and also depresses the voluntary motor nerves, we submit that it is more probable that the slowing and weakening of the heart is due to the action of atropia on the excito-motory apparatus.

Experiments to ascertain how atropia antagonises pilocarpine and muscarin.

As has been repeatedly shown, atropia removes or prevents in a great degree the slowing and weakening effect of pilocarpine and muscarin on the heart. Through which structures is this antagonism effected? It is usually held that as atropia paralyses the extra- and intra-cardiac inhibitory apparatus, it is probable that pilocarpine slows and weakens the heart by stimulating this inhibitory apparatus, and that atropia by paralysing this apparatus prevents the action of pilocarpine. If this theory is correct then atropia should completely prevent or remove the effects of pilocarpine. We made some experiments to ascertain how far atropia does antagonise the effects of pilocarpine. We used a two per cent. solution of pilocarpine nitrate, and a solution of the same strength of atropia sulphate. We find that this solution of atropia sulphate does antagonise the action of pilocarpine, but only to a limited extent, improving the strength but not always the frequency of the beats. We give our results in Table III.

We made a corresponding series of experiments with extract of *Amanita muscaria,* which is supposed to slow and weaken the heart in the same way as pilocarpine.

We used a 1 in 5 solution of extract of muscaria and a two per cent. solution of atropia sulphate. In these experiments the antagonism was far from complete, for though the atropia increased the strength and frequency of the beats, it did not restore them to their normal frequency or strength.

We give the results of these experiments in Table IV.

Table III.

Date.	After application of pilocarpine the pulsations fell	The pulsations became	After the application of atropia the pulsations were	The pulsations became	Heart recovered in	Pulsations continued	
Jun. 10	44 to 32	very weak	16	rather stronger	7 hours	28 hours	
„ 14	44 to 20	weak	16	decidedly improved but remained weak	recovered frequency in 6 hours but grew weaker		
„ 17	56 to 36	rather weak	28	rather improved	recovered frequency and strength in 2 hours	18 hours	
„ 17	56 to 28	very weak	24	improved but continued weak	never recovered	2 hours	
„ 18	56 to 28	weak	36	became stronger			½ p. c. small quantity

Table IV.

Date.	After application of muscarin the pulsations fell	The pulsations became	After the application of atropia the pulsations were	Pulsations became	Heart recovered in	Pulsations continued	Strength of solution of extract of amanita muscaria
Jun. 10	42 to 12	weak	24	fair	5 hours	42 hours	2 p. cent.
„ 18	76 to 0	stopped	24	weak	in frequency in 4 hours		1 in 5
„ 21	44 to 8	weak	24	rather weak	recovered in frequency in 2 hours; never recovered in force		1 in 5
„ 21	48 to 2	weak	28	rather weak	recovered in frequency and force in 2 hours		1 in 5

At first sight it might be thought that these results are fatal to the explanation usually accepted, but a little consideration will show that this is not the case.

It must be remembered that we used a strong solution of atropia (two per cent.), and the result was that in the one case we replaced the effects of pilocarpine with those of atropia, and in the other the effects of muscarin by those of atropia. After the application of the atropia the condition of the heart as regards both strength and frequency was similar to that occurring when atropia alone is applied to the heart. It may then be said that pilocarpine (and muscarin) slow, weaken, or arrest the heart by stimulating the cardiac inhibitory apparatus, and that atropia paralysing this apparatus obviates the effect of pilocarpine; but if a strong solution of atropia be used, it not only paralyses the inhibitory apparatus, but the atropia weakens and paralyses the excito-motory apparatus, and so the heart remains weak and slow.

At this point of our investigations it occurred to us that as atropia not only paralyses the inhibitory apparatus but probably also weakens or paralyses the excito-motory apparatus, two theories regarding the antagonism of atropia for pilocarpine are feasible.

Pilocarpine may have a chemical affinity for the inhibitory apparatus, and by combining with this nervous structure it may increase its functional activity, or stimulate it as it is termed. Atropia having a stronger chemical affinity for this nervous structure displaces the pilocarpine and paralyses the inhibitory apparatus. But another view is feasible, for we have seen that probably atropia weakens or paralyses the excito-motory apparatus of the heart. Pilocarpine and muscarin may possess a chemical affinity for the cardiac excito-motory apparatus, and in combining with its nervous elements weakens or paralyses this apparatus. Atropia may possess a stronger chemical affinity for the nerve elements of the excito-motory apparatus, and so displace the pilocarpine and replace the weakening effect of the pilocarpine for the lesser weakening effect of atropia. That in fact both act on the same structure and in the same way, but that atropia in virtue of its greater affinity displaces the pilocarpine or muscarin. Consequently the atropia prevails, and we get what we may term an atropia heart instead of a pilocarpine or a muscarin heart.

Our subsequent experiments were devised to ascertain which of these theories is the more feasible.

We first experimented to learn if muscarin paralyses the excito-motory apparatus or the muscular substance of the heart. At the suggestion of Dr Sanderson we tested the effect of muscarin on the excised ventricle with the auriculo-ventricular groove attached; this portion of the heart being, according to the accepted view, free from any inhibitory nervous apparatus and containing only excito-motory.

We give in Table V. a record of our experiments.

Table V.

Date.	Before the application of muscaria extract the heart beat per minute	After the application of muscaria the beats rose to	The acceleration continued	The heart stopped after application of muscaria in	After the application of atropia the heart began to beat in	The pulsation rose to	Continued beating
July 2	7 good	44	3 minutes	4 minutes	1 minute	18 good	9 hours
Carbonate of soda used.							
,, 4	42 good		not quickened	7 minutes	1 minute	36 good	3 hours
,, 4	12 good		not quickened	almost stopped in 5 minutes and fell to 6 beats per min.	grew stronger in 5 minutes and became fairly strong	continued at 6 beats per min.	3 hours
,, 5	4 good	20		11 minutes	1 minute	20 good	1 hour

We placed the excised ventricle in a watch-glass, containing an equal quantity of the frog's blood and a one per cent. solution of common salt. We employed a solution of extract of amanita muscaria 1 in 5, and a 1 in 400 solution of sulphate of atropia.

In these four experiments we found that after the ventricle was stopped by extract of muscaria, on the application of atropia pulsations returned, became good, and continued on an average four hours.

The solution of amanita muscaria has a faint acid reaction, and we thought it possible that the arrest of the contractions might be due to the acid reaction. In the three last experiments therefore, when the heart was stopped we added a two per cent. solution of carbonate of soda, but without exciting any contractions; we then added the solution of sulphate of atropia.

On many occasions we found on excising the ventricle with the auriculo-ventricular groove attached, that the contractions soon ceased, but could readily be restored for a few minutes by simple mechanical stimulation of the ventricle, as by pinching or pressing with the forceps.

We tested the antagonism of muscarin and atropia on some of these hearts, and find that extract of muscaria paralyses them whilst atropia restores them to their former condition. We used in these experiments a solution of the extract of amanita muscaria 1 in 5, and a 1 in 400 solution of sulphate of atropia. We now record some of our experiments.

July 8th.
7.45. Ventricle excised.
8.11. Stopped, but mechanical stimulation restores the contraction for a short time. Muscarin applied.
8.14. Very slight contraction on mechanical stimulation.
8.15. No contraction on stimulation. Atropia applied.
8.17. A few seconds after mechanical stimulation contractions began.
8.18. 16 fair beats per minute.
8.25. 20 good beats ,,
8.27. 12 rather weaker.
8.32. 6 feeble beats.
8.42. Mechanical stimulation, beats strengthened at once.
8.44. 22 fairly good.
8.52. 10 feeble. Mechanical stimulation strengthened beats.
10.30. On mechanical stimulation there followed one feeble beat.

July 9th.
7.43. Ventricle excised.
7.46. 20 good.
7.50. Stopped. Mechanical stimulation applied.
7.51. 32 good, and continued beating for a minute.
7.52. Muscarin extract applied.
7.54. Mechanical stimulation.
7.56. 35 per minute.
7.59. Stopped.
8. Mechanical stimulation without effect.
8.1. Sulphate of atropia applied.
8.3. Began to beat spontaneously.
8.4. 20 per minute, good.
8.8. Same.
8.17. 12 per minute, decidedly weaker.
8.25. Just stopped. Mechanical stimulation.
8.27. 4 per minute, rather feeble beats.
8.32. 2 per minute, feeble.
8.39. Stopped.

July 10th.
7.34. Ventricle excised.
7.40. Stopped.
7.45. Applied a galvanic shock, followed by a succession of beats lasting about ¼ minute.

7.47. Muscarin extract applied.
7.50. Galvanism applied. Contractions lasting for 15 seconds. Muscarin extract reapplied.
7.52. Spontaneous contractions for about 15 seconds.
7.54. Galvanism applied. 17 contractions in the following ½ minute.
7.57. Galvanism applied. No contraction.
7.59. Sulphate of atropia applied.
8. Spontaneous contractions began.
8.1. 28 feeble contractions per minute.
8.3. 22 per minute, much stronger.
8.6. 20 ,, fair beats.
8.14. 13 ,, good.
8.20. 13 ,, good.

July 16th.
2.45. Heart excised and placed in 1 p.c. solution of chloride of sodium.
2.49. 16 very good.
2.57. Stopped.
2.59. Mechanical stimulation, 36 per minute.
2.59½. Stopped again. Mechanical stimulation.
3. 36 per minute.
3.1. Stopped.
3.3. Extract of amanita muscaria applied.
3.7. Mechanical stimulation. 40, weaker, per minute.
3.8½. Stopped.
3.11. Mechanical stimulation. 36, very weak, per minute.
3.16. Mechanical stimulation. 40, only just visible, per minute.
3.20. Mechanical stimulation. Contractions only just visible.
3.20½. Sulphate of atropia applied.
3.23. Mechanical stimulation. 4, much stronger, per minute.
3.25. Mechanical stimulation. 24 fair beats per minute.
3.27. Mechanical stimulation. 44 per minute, strong beats.
3.29½. Stopped.
3.36. Mechanical stimulation. 16 per minute, good.
3.36½. Stopped.
3.39. Mechanical stimulation. 16 good per minute.
3.40. Stopped.
3.41½. Mechanical stimulation. 40 per minute, very good.
3.43. Stopped.
3.46. Mechanical stimulation. 1 feeble beat.
3.48. Mechanical stimulation. No effect.

In the previous experiments the heart soon stopped beating; this we ascribed to the heat of the room, and we determined to keep the heart cool, so we placed the watch-glass containing the heart on a block of ice.

July 18.
7.52. Heart excised.
7.54. 4 prolonged contractions per minute, each contraction lasting 6 to 7 seconds.

8. 2 per minute.
8.4. Stopped.
8.5. Mechanical stimulation, one prolonged contraction on touching any part of ventricle.
8.8. Muscarin applied.
8.11. Mechanical stimulation produced one strong contraction.
8.13. Same.
8.17. ,,
8.30. ,,
8.32. 7 spontaneous contractions per minute.
8.36. Spontaneous contractions ceased.
8.37. On mechanical stimulation one good contraction.
8.41. Same.
8.42. Muscarin re-applied.
8.48. After mechanical stimulation no contraction.
8.50. Same. Sulphate of atropia applied.
8.52. After mechanical stimulation no contraction.
8.56. Same.
10.40. Mechanical stimulation. 10 fair beats.
10.47. Mechanical stimulation. 17 beats in half a minute.

July.
7.41. Heart excised.
7.46. 5 very strong contractions per minute, each contraction lasting about 7 seconds.
7.53. Stopped. Mechanical stimulation excited one strong contraction.
7.56. Muscarin applied.
7.59. Mechanical stimulation. 5 strong contractions per minute.
8.9. Contractions ceased.
8.12. Mechanical stimulation. One doubtful contraction.
8.15. Mechanical stimulation. No contraction.
8.17. Sulphate of atropia applied.
8.20. Mechanical stimulation. No effect.
8.22. Spontaneous contractions, 3 very long, but not strong, contractions.
8.28. 3 per minute, good contractions.
8.34. 4 strong contractions per minute.
8.39. 5 ,, ,,
10.20. Stopped. Mechanical stimulation. No effect.

In both these experiments we noticed that before the application of extract of muscarin mechanical stimulation excited only one contraction, but soon after the action of muscaria the heart continued contracting after mechanical stimulation for several minutes. The muscarin indeed at first appeared to act as a 'stimulant.'

In all these experiments extract of muscarin arrested the pulsations of the ventricle, and as the portions we used were free from any inhibitory apparatus, we must conclude that muscarin in part at least arrests the heart by paralysing either its excito-motory apparatus, or its muscular substance, or both.

After the heart was paralysed by extract of muscarin we added a small quantity of a weak solution of atropia, and in every instance pulsatile activity returned, indeed often the heart beat spontaneously, but when this did not occur, mechanical or galvanic stimulation excited contraction, and in most instances strong contractions; in other words, atropia antagonises the action of muscarin on the ventricle with the auriculo-ventricular groove attached. As this portion of the heart is free from an inhibitory apparatus, it is evident that the paralysis is not due to stimulation of the inhibitory apparatus by muscarin, and the antagonism of course is not due to the atropia paralysing the inhibitory apparatus, and so obviating the effect of muscarin.

Muscarin, we have concluded from these experiments, paralyses the ventricle either by paralysing the excito-motory apparatus, or the muscular substances, or both, and the antagonism by atropia must be effected by its influence on one or both of these structures.

In a previous section of this paper we have shown that atropia, like muscarin, paralyses the heart by its influence on the excito-motory apparatus, or the muscular substance of the heart. How then is the antagonism effected on the ventricle by these two substances, which both paralyse it?

We suggest it depends on a chemical displacement. Atropia has a stronger chemical affinity for the excito-motory apparatus or muscular substance than muscarin, but at the same time the paralysing power of atropia is much weaker than that of muscarin; or in other words, muscarin combines with the nervous structure of the excito-motory apparatus or muscular structure, and paralyses the heart. Atropia, from its stronger affinity for these structures, displaces the muscarin, but as its paralysing action is slight, the heart contracts again.

We next tested the antagonism between atropia and extract of muscaria on the ventricle without the auriculo-ventricular groove attached to it. The ventricle then gives one contraction on each stimulation. We used solutions of the same strength as in the previous experiments. We give a record of some of our observations.

8.10. Applied muscarin.
8.13. No contraction on stimulation. Atropia applied.
8.15. No contraction on mechanical stimulation.
8.25. A good contraction on stimulation.
8.29. „ „
8.37. „ „

8.45. A good contraction on stimulation.
8.53. ,, ,,
10.30. No contraction on mechanical stimulation.

In the following experiments we placed the cut-out apex in a watch-glass surrounded by ice, but the watch-glass was not in contact with the ice. We were careful to avoid the auriculo-ventricular groove, and in the second experiment we removed only the apex, leaving the greater part of the ventricle behind.

1.59. Heart excised.
2.2. Mechanical stimulation followed by a single strong contraction.
2.6. ,, ,, ,,
2.9. ,, ,, ,,
2.10. Muscarian applied.
2.15. Mechanical stimulation. One distinct contraction.
2.24. ,, One feeble contraction.
2.28. ,, ,,
2.34. ,, ,,
2.37. ,, No contraction. Atropia, 1 in 400, applied.
2.40. ,, A distinct contraction.
2.44. ,, A good contraction.
2.49. ,, ,,
2.55. ,, 12 good contractions per minute.
2.58. Contractions ceased.
3.5. Mechanical stimulation. One fair contraction.
3.10. ,, ,,
3.17. ,, ,,
3.22. ,, ,,
3.29. ,, ,,

April 22.
2.19. Apex excised.
2.20. Mechanical stimulation followed by a single strong contraction.
2.26. Same.
2.29. Same. Extract of amanita muscaria applied.
2.33. Mechanical stimulation. No contraction.
2.34. Atropia applied.
2.37. Mechanical stimulation. No contraction.
2.40. ,, ,,
2.50. ,, One good contraction.
2.55. ,, ,,
3.5. ,, ,,
3.10. ,, ,,
3.14. Two spontaneous good contractions per minute.
3.17. ,, ,,
3.22. Three ,, ,,
3.29. ,, ,, ,,

These experiments show that extract of muscaria is poisonous to the apex of the ventricle, which contains neither inhibitory nor excito-motory ganglia, in fact that extract of muscaria paralyses the muscular substance of the heart; and they further show that atropia antagonizes the action of muscarin on the muscular substance of the ventricle.

In our two last experiments we obtained a curious result. Although only the apex of the ventricle was removed, and while, before the drugs were applied, mechanical stimulation excited only one contraction; yet after atropia had been applied for some minutes, spontaneous contractions began and persisted for fifteen minutes.

In the antagonism of atropia for muscarin we have an example of a remedy which slows and weakens the heart antagonizing the action of another remedy which also slows and weakens the heart. In the previous number of this Journal we have given another example of the same action, for we have shown that pilocarpine antagonizes the action of muscarin, and these two remedies both slow and weaken the heart; and we found that pilocarpine is almost as powerful an antagonist for muscarin as atropia.

We now suggest the same explanation for the antagonism of pilocarpine for muscarin as we have offered for the antagonism of atropia for muscarin; namely, that pilocarpine has a stronger affinity for the cardiac structures than muscarin, but pilocarpine is a far feebler paralyser than muscarin. On account of its stronger affinity, pilocarpine, when added to a heart weakened or arrested by muscarin, displaces the muscarin, and taking its place substitutes its own action for that of muscarin, and having a weaker paralysing effect, the heart's contractions return, or if only 'slowed' and weakened, grow stronger and more frequent.

If this view is correct, it is obvious that whilst pilocarpine will antagonize muscarin, muscarin should not antagonize pilocarpine. We have made some experiments to test if muscarin does antagonize pilocarpine.

We performed a series of comparative experiments. We tested the action of

(i) nitrate of pilocarpine on the heart,

(ii) nitrate of pilocarpine followed by extract of muscarin,

(iii) nitrate of pilocarpine followed by a 1 per cent. solution of nitrate of muscarin obtained from amanita muscaria,

(iv) nitrate of pilocarpine followed by a 1 in 120 solution of sulphate of atropia.

Table VI. Antagonism of ext. of amanita muscaria to pilocarpine.

Date.	After application of pilocarpine pulsations fell	After application of pilocarpine heart became	After application of muscarin pulsations	After application of muscarin pulsations became	Pulsations continued
Feb. 18	in 2 min. from 40 to 28 and fell in 2 hours to 16	very weak indeed in two hours	fell to 4 in 6 min.	even weaker	16 min. after application of muscarin.
,, 19	in 1 min. from 44 to 28	very weak	remained same	rather weaker	beating at 32 badly 14 hours after application.
	in 2 ,, 64 to 40	,,	,,	weaker	continued beating more than 4 hours.
,, 21	in 1 ,, 48 to 24	,,	,,	weaker	beating at 32, 8 hours after.
,, 21	in 1 ,, 52 to 24	weaker	fell to 12 in 3 min.	weaker	

Table VII. Nitrate of muscarin used instead of extract.

Feb. 18	in 2 min. from 44 to 36 and in 2 hours to 28	very weak, almost stopped	no increase	no improvement	1 hour 16 min. after muscarin.
,, 19	in 1 min. from 48 to 32 and ultimately to 28	very weak	rose from 28 to 36	no improvement	beating weakly at 8 fourteen hours after.
,, 19	in 2 min. from 44 to 16	almost stopped	fell to 12 in 4 min.	worse	stopped in an hour.
,, 21	in 3 ,, 56 to 28	not very weak	rose to 40 in 7 min.	remained same	beating at 28 good beats eight hours after.
,, 21	in 1 ,, 60 to 28	not very weak	rose to 40 in 6 min. & fell in 1 hr to 10	remained same	
,, 21	in 11 ,, 48 to 20	very weak	fell from 20 to 12	weaker	20 min. after application of muscarin.
,, 21	in 13 ,, 52 to 12	not very weak	remained same	stronger	in 4 hours beat badly at 8 per minute.

Before making this series we thought it better to test the power of our 1 per cent. solution of nitrate of muscarin on the frog's heart.

Exp. i. The heart's beats slowly fell from 40 and stopped in 70 minutes.

Exp. ii. The heart's beats fell slowly from 34 and stopped in 70 minutes.

Exp. iii. The heart's beats fell slowly from 40 and stopped in 73 minutes.

Exp. iv. The heart's beats fell slowly from 36 and stopped in 53 minutes.

We give the results of our observations in Tables VI., VII., and VIII.

Table VIII. Antagonism of atropia to pilocarpine.

Date.	After application of pilocarpine pulsations fell	After application of pilocarpine pulsations became	After application of atropia pulsations rose	After application of atropia pulsations became	Pulsations continued
Feb. 8	in 1 min. from 48 to 0	stopped	in 3 min. to 40	good	was beating well at 32 four hours and a half afterwards, when observations were discontinued
,, 18	in 2 min. from 40 to 28 in 2 hours fell to 12	very weak	in 2 min. to 18 and in 2 hours to 28	very good	14 hours after 24 very good

Action of pilocarpine on heart. 10 per cent. solution.

Feb. 8. The heart beating at 44 fell in 11 minutes to 24, and in four hours and a half to 8 and became very weak.

,, 18. The heart beating at 36 fell in 5 minutes to 20, very weak; four hours after it beat 24, very weak; fourteen hours after it had stopped.

In the four experiments in which we applied extract of muscaria after pilocarpine we obtained no antagonism, but on the contrary in every instance the pulsation became weaker.

Of the seven experiments with nitrate of muscarin: in five the strength of the beats remained the same; in one the beats became weaker; in one they became stronger. In three the beats became more frequent after the application of muscarin, but we doubt if this is due to the muscarin, and believe it would have occurred without it; for we find that pilocarpine when first applied greatly slows the heart, and after a few minutes the beats become more frequent again.

In May we resumed our investigation.

We applied some 1 in 10 solution of nitrate of pilocarpine to a heart beating at 20. In 30 minutes the auricles stopped and the ventricle beat very feebly at 22, only the base of the ventricle contracting. We then applied a very small quantity of a 1 in 50 solution of extract of amanita: in five minutes we applied a little more. The left auricle at once commenced contracting feebly; in fifteen minutes from the first muscarin application it contracted well. In sixteen minutes both auricles contracted well at 20, and the ventricle had grown a little stronger. In eighteen minutes the ventricular contraction had greatly improved, and in twenty-one minutes both auricles and the ventricle contracted as well as before the experiment was begun. In thirty-five minutes the ventricle and the right auricle stopped. We then reapplied the muscarin solution, and the left auricle at once grew much weaker, so that its contraction only caused a quiver in its walls, whilst the other auricle and the ventricle remained motionless. We then waited five minutes, and applied a 1 per cent. solution of sulphate of atropia to the heart, and in one minute all the cavities began contracting, and in two minutes the contractions were good.

In this experiment there was a distinct antagonism, lasting however only about half an hour, and on the reapplication of muscarin the heart all but ceased, showing that if too much muscarin be applied, so far from antagonizing pilocarpine it assists its action. The greatly superior antagonistic action of atropia is shown in this experiment, for when only the left auricle contracted very faintly, atropia restored vigorous pulsations in all the cavities.

Next day we repeated the experiment, but employed extract of jaborandi instead of pilocarpine; after the application of the extract to the heart, beating at 56, in thirty-three minutes the ventricle stopped and the left auricle beat feebly at 20. We then applied a very small quantity of the 1 in 50 solution of extract of amanita. This greatly increased the strength of the contractions of the left auricle, but the right auricle and the ventricle remained motionless. We waited twenty minutes without any change, and again applied muscaria solution, this time freely, when immediately the contractions of the left auricle grew much feebler and in four minutes had all but stopped. We then applied a very small quantity of a 1 per cent. solution of atropia, and in three minutes all the cavities contracted well.

This experiment, like the last, shows that muscarin is in a small degree antagonistic to pilocarpine, though in this case it only strengthened the beats of the left auricle. On the addition of a larger quantity of muscarin this cavity grew much weaker.

May 27. We applied three times, at about five minutes interval, some 1 in 10 solution of nitrate of pilocarpine. This reduced the ventricle's contractions from 48 to 1, and the auricular to 20, and greatly weakened them. Some 1 in 50 of extract of amanita muscaria was applied twice, at 13 minutes interval, but the heart's contractions became still weaker. A little of a 1 per cent. solution of atropia sulphate was then applied, and in eleven minutes the heart beat 22 good beats per minute.

These experiments show that in the great majority of cases, muscarin does not in any degree antagonize the action of pilocarpine, but on the contrary the pulsations grow weaker. In a few instances, however, the pulsations grew stronger and more frequent for a short time, but the pulsations are at once arrested if the muscarin solution is applied a second time at all freely.

Summary of our Conclusions.

I. Sulphate of atropia topically applied is almost as powerful a paralyser of the heart as pilocarpine.

II. Atropia paralyses either the excito-motory cardiac apparatus, or the cardiac muscle, or both these structures.

III. Extract of muscaria paralyses the cardiac muscular substance and probably the excito-motory apparatus.

IV. In part at least, atropia antagonizes the action of muscarin by possessing for the cardiac structures (excito-motory apparatus and cardiac muscle) a stronger affinity than muscarin, whereby atropia, a cardiac paralyser, replaces muscarin, also a cardiac paralyser: but atropia has a far weaker paralysing action than muscarin: hence when atropia replaces muscarin in the cardiac structures, the powerful paralysing effect of muscarin is replaced by the far weaker paralysing effect of atropia, and so a heart weakened or arrested by muscarin becomes strengthened, or commences beating again, on the addition of atropia.

V. Pilocarpine, which weakens or arrests the heart, also antagonizes the action of muscarin, which still more powerfully paralyses the heart. We suggest the same explanation for this antagonism as for the antagonism of atropia for muscarin, namely, that it is due to chemical displacement. Pilocarpine having a stronger affinity for the excito-motory apparatus and for the muscular substance, displaces muscarin and substitutes its own weaker paralysing action for that of muscarin.

VI. Our experiments do not show that the antagonism between atropia and muscarin is not in part due to their antagonism on the inhibitory apparatus. In which case atropia, having a stronger chemical affinity for the structures of inhibitory apparatus, displaces muscarin and substitutes its paralysing action for the stimulant action of muscarin.

In our previous experiments we noticed that though atropia markedly antagonizes the action of pilocarpine, yet the heart soon ceased to beat, much sooner than after the application of muscarin followed by atropia; and we made some experiments to ascertain how soon the heart stops after the effect of pilocarpine has been antagonized by atropia. We give our results in Table IX. We employed a 2 per cent. solution of nitrate of pilocarpine and a 1 per cent. solution of sulphate of atropia.

Table IX.

Date.	Heart's beats reduced by pilocarpine from	Pulsations became	After application of atropia pulsations rose to	Pulsations became	Heart continued beating
May 28	20 to 4	weak	20	good	5 hours
„ 28	48 to 16	weak	28	good	8 hours
„ 29	48 to 20	rather weak	28	good	7 hours
„ 29	52 to 20	rather weak	36	good	9 hours

CONCERNING THE ACTION OF COMMON SALT, SULPHATE OF ATROPIA, BROMIDE OF CONIA AND SULPHATE OF NICOTINE ON MUSCULAR IRRITABILITY.

By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London,* and E. A. MORSHEAD, L.R.C.P., *Assistant to the Professor of Medicine at University College, London.*

In Vol. I. of this Journal we published a paper showing that chloride of potassium is a powerful protoplasmic poison, speedily destroying function in the cord, nerves and muscles. We made two series of experiments. In the first, muscular contractility persisted on an average four times longer, and in the second, twelve times longer in the unpoisoned than the poisoned frogs.

We have also shown that tartar emetic, arsenious acid, aconitia, hydrocyanic acid, are likewise powerful protoplasmic poisons, and we determined to pursue this subject, and to test the action of other substances on muscular irritability, following the same plan we adopted in our previous experiments, namely, to ascertain how long muscular irritability continued in the poisoned frogs compared to brainless frogs with the circulation arrested by excision of the heart.

We first experimented with common salt.

We were obliged to conduct these experiments somewhat differently from those with chloride of potassium, for this salt quickly paralyses the heart, whilst chloride of sodium has a far less depressing action on the heart. We found indeed that after the injection of the salt the heart continued beating well, and so to compare its action on the muscles with our unpoisoned test frogs with extirpated heart, we first poisoned the frog by injecting the solution of common salt, and then after allowing time for the absorption of the salt, we cut away the heart. Thus the only difference between the poisoned frog and the test frog was the presence of the excess of common salt from our injection.

In these experiments we used "brainless frogs," that is, before experimenting we cut across the occipito-atlantoid membrane and destroyed the brain by thrusting a wooden peg into the cavity of the skull.

We give the results of our experiments in Table I.

Table I. Influence of common salt on muscular contractility.

Date.	Dose in grains.	Weight of frog in grammes.	Proportionate dose.	Heart cut after the injection under the skin of the back.	Muscular contractility ceased in			Strength of solution.	
					anterior limbs.	thighs.	lower leg muscles.		
Oct. 11	1	38	$\frac{1}{585}$	24 min.	36 hours	41 hours	59 hours	1 in 5	Injection administered before the brain destroyed.
„ 11	1	26	$\frac{1}{400}$	25 min.	19 hours	28 hours	33 hours	1 in 5	
„ 11	1	36	$\frac{1}{554}$	26 min.	19 hours	33 hours	59 hours	1 in 5	
		Average	$\frac{1}{513}$		24·6 hours	34 hours	50 hours		
Feb. 10	1	24	$\frac{1}{369}$	one hour	52 hours	58 hours	78 hours	1 in 5	Brain destroyed before the hypodermic injection.
„ 11	2	46	$\frac{1}{354}$	one hour	34 hours	35 hours	60 hours	1 in 5	
„ 13	1	31	$\frac{1}{477}$	half an hour	55 hours	53 hours	78 hours	1 in 5	
		Average	$\frac{1}{400}$		47 hours	49 hours	72 hours		
Test Frogs: brain destroyed and heart cut across.									
Oct. 11					30 hours	41 hours	74 hours		
„ 11					36 hours	51 hours	76 hours		
„ 11					19 hours	36 hours	82 hours		
				Average	28 hours	42·6 hours	77 hours		
Feb. 11					70 hours	76 hours	98 hours		
„ 13					68 hours	79 hours	96 hours		
				Average	69 hours	77 hours	97 hours		

Table III. Effect of sulphate of atropia on muscular irritability.

Date. 1878.	Dose in grains.	Weight of animal in grammes.	Proportionate dose.	Paralysis complete.	Muscular irritability ceased in			Strength of solution.	Heart divided after poisoning.
					anterior limbs.	thighs.	lower leg muscles.		
Oct. 5	1	35	$\frac{1}{539}$	14 min.	29 hours	25 hours	50 hours	1 in 5	36 min.
,, 5	1	31	$\frac{1}{477}$	2 min.	32 hours		36 hours	1 in 5	37 min.
,, 5	1	28	$\frac{1}{431}$	18 min.	36 hours	32 hours	80 hours	1 in 5	34 min.
		Average	$\frac{1}{482}$	11 min.	32·3 hours	28·5 hours	55 hours		
Test Frogs: brain destroyed and heart cut through.									
Oct. 5					30 hours	50 hours	60 hours		
,, 5					45 hours	60 hours	80 hours		
,, 5					42 hours	40 hours	80 hours		
				Average	39 hours	50 hours	73 hours		

These large doses of common salt therefore exert a slight action on muscular irritability. We extract the averages in Table II.

Table II. Frogs poisoned with common salt.

Date.	Anterior limbs.	Thighs.	Lower leg muscles.
Oct. 11	24 hours	34 hours	50 hours
Feb.	47 hours	49 hours	72 hours
Test frogs.			
Oct.	28 hours	42 hours	77 hours
Feb.	69 hours	77 hours	97 hours

Muscular irritability therefore lasted about half as long again in the test as in the salt frogs, whilst with the same relative dose of chloride of potassium, muscular irritability persisted four and twelve times longer in the test than in the potash frogs.

The Action of Sulphate of Atropia on muscular irritability.

We performed these experiments in the same way as those with common salt, that is to say, we injected the atropia solution under the skin and then allowed time for it to be distributed through the circulation; we then cut through the heart. We at the same time prepared some test frogs by cutting through the heart.

We give our results in Table III.

We extract the averages and give them in Table IV.

Table IV. Frogs poisoned with atropia.

Date.	Proportionate dose.	Muscular irritability ceased in		
		anterior limb.	thighs.	lower leg muscles.
Oct.	$\frac{1}{482}$	32 hours	28 hours	55 hours
Test frog.				
Oct.		39 hours	50 hours	73 hours

By comparing Table IV. with Table II. giving the results of our observations on common salt, it will be seen that in about the same proportionate dose, atropia has no greater influence on muscular irritability than common salt.

We then tested the action of bromide of conia on muscular irritability, performing experiments as with common salt and atropia. We give our results in Table V.

Table V. Action of bromide of conia on muscular irritability.

Frogs first poisoned and when paralysed the heart cut through.

Date.	Dose in grains.	Weight of animal in grammes.	Proportionate dose.	Paralysis complete.	Muscular irritability ceased in			Strength of solution.
					anterior limbs.	thighs.	lower leg muscles.	
Apr. 11	1	29	$\frac{1}{447}$	13 min.	20 hours	20 hours	40 hours	1 in 5
,, 11	1	30	$\frac{1}{462}$	12 min.	34 hours	38 hours	63 hours	1 in 5
,, 11	1	28	$\frac{1}{432}$	11 min.	24 hours	20 hours	32 hours	1 in 5
		Average	$\frac{1}{447}$	12 min.	26 hours	26 hours	45 hours	

Test frogs. Brainless with heart cut through.

Date.	Dose in grains.	Weight of animal in grammes.	Proportionate dose.	Paralysis complete.	anterior limbs.	thighs.	lower leg muscles.	Strength of solution.
Apr. 11					33 hours	33 hours	64 hours	
,, 11					24 hours	39 hours	65 hours	
,, 11					43 hours	53 hours	52 hours	
					33 hours	41·6hours	60 hours	

We give the averages in Table VI.

Table VI. Frogs poisoned with bromide of conium.

Date.	Proportionate dose.	Muscular irritability ceased in		
		anterior limbs.	thighs.	lower leg muscles.
April	$\frac{1}{447}$	26 hours	26 hours	45 hours
Test frog.				
April		33 hours	41 hours	60 hours

Bromide of conium is therefore a very weak muscle poison, no more powerful in this respect than common salt; an enormous dose of $\frac{1}{400}$ of the weight of the animal, equivalent to about five ounces of the poison to a man weighing 150 lbs., being required to produce even a slight effect.

We next tested the action of nicotine on muscular irritability.

As in our previous experiments we first destroyed the brain and then injected the solution of nicotine under the skin of the back, and after about an hour had elapsed we cut away the heart to arrest the circulation. At the same time we prepared some test frogs by destroying their brains and cutting away their hearts.

We used a 1 per cent. solution of nicotine.

We give the results of our experiments in Table VII.

Table VII. The action of nicotine on muscular irritability.

Date.	Weight in grammes.	Dose in grains.	Proportionate dose.	Muscular irritability ceased in		
				anterior limbs.	thighs.	lower leg muscles.
Feb. 17	28	$\frac{1}{20}$	$\frac{1}{8624}$	16 hours	32 hours	79 hours
,, 17	38	$\frac{1}{20}$	$\frac{1}{11704}$	13 hours	35 hours	30 hours
,, 21	45	$\frac{1}{20}$	$\frac{1}{13860}$	24 hours	35 hours	75 hours
,, 21	37	$\frac{1}{20}$	$\frac{1}{11396}$	33 hours	56 hours	74 hours
,, 21	37	$\frac{1}{20}$	$\frac{1}{11396}$	31 hours	56 hours	94 hours
		Average	$\frac{1}{11396}$	23·4 hours	43 hours	70 hours

Brainless test frogs with heart cut through.

Feb. 17				36 hours	49 hours	75 hours
,, 17				89 hours	102 hours	126 hours
,, 19				58 hours	80 hours	122 hours
,, 22				61 hours	81 hours	120 hours
				61 hours	78 hours	111 hours

Nicotine we see has a slight depressing action on muscular irritability, and is more powerful in this respect than common salt, sulphate of atropia and bromide of conium; muscular contractility lasting nearly twice as long in the unpoisoned as in the poisoned frogs. In another series of experiments we employed a much larger dose of nicotine, and certainly increased the paralysing effect on muscular irritability, but only in a slight degree, as can be seen on referring to Table VIII.

Table VIII. The action of nicotine on muscular irritability.

Date.	Weight in grammes.	Dose in grains.	Proportionate dose.	Muscular irritability ceased in		
				anterior limbs.	thighs.	lower leg muscles.
Feb. 25	35	½		15 hours	32 hours	52 hours
,, 25	25	½		15 hours	21 hours	45 hours
				15 hours	26·5 hours	48·5 hours
Brainless test frogs with heart cut through.						
Feb. 25				32 hours	74 hours	99 hours
				33 hours	56 hours	79 hours
				32·5 hours	65 hours	89 hours

Nicotine is therefore a muscle poison, and we shall get some idea of its action when we reflect that the dose we administered to the frogs is equal to about 105 minims to a man weighing 150 lbs.

We made this series of experiments to test the feasibility of a theory, that medicines are poisons to all protoplasm; that some like potash appear to be equally poisonous to all forms of protoplasm, nerve-cells, nerve-fibres, muscle, &c.; that others, like arsenic, antimony and aconite, whilst powerfully poisonous to all protoplasm, are especially poisonous to some; that others again have only a comparatively feebly poisonous action on some tissues and a very powerful one on other tissues—as for instance, nicotine, which cannot be considered a powerful muscle poison, but is a very powerful poison for the spinal cord and for motor nerves; that in others again the poisonous action on some tissues is of the feeblest character, whilst their action is powerful on others—as for instance, atropia, conia, substances having a powerful poisonous action on many nervous structures, but are no more poisonous to muscle than common salt.

It may be said, that admitting that medicines have an affinity for all nitrogenous tissue (protoplasm), it must be incorrect to speak of them as protoplasmic poisons, for some substances cause an increased evolution of nerve or muscle force, and others heighten the conductivity of the nerves, and that we must here suppose that the substance uniting with the nerve or muscle tissue forms a compound which more readily undergoes those chemical changes leading to the evolution of force. For instance, strychnia, by its action on the cord, leads on stimulation to the discharge of a greater amount of nervous impulses and over an extended area, and that we must conclude that strychnia forms a compound with the nervous structure more unstable, or more explosive than simple nerve-tissue, and hence on stimulation there results a greater discharge of impulses, and over an extended area of the cord. Digitalis again heightens either the conductivity of the vagi or the inhibitory apparatus in the heart, and also the excito-motory apparatus of the heart. Here, it may be said that the active principle of digitalis forms a compound with the nervous elements of the vagi, so that impressions are more readily conducted, or that it combines with the cells of the intra-cardiac inhibitory apparatus, so that a stimulus causes an increased evolution of nerve-force. The same explanation may be applied to the action of atropia on the excito-motory apparatus of the heart.

We however suggest another view, which we have fully propounded elsewhere, in respect of the action of strychnia on the spinal cord.

All the tissues are under the influence of a control called inhibition, resistance, &c., by which their functional activity is regulated: if this is heightened, functional activity is depressed; if it is weakened, functional activity is increased. Now we suggest that drugs which are spoken of as stimulants or excitants, are really paralysers of the structures that offer this resistance, or exert this inhibition, that in fact they combine with those structures which inhibit or resist, and weaken or destroy their controlling limiting action, and so lead to increased evolution of forces, or a more ready transmission of an impression.

Some poisons have an affinity, and so a paralysing action on both the structures which inhibit and those through whose decomposition functional energy is developed, but most on the inhibitory structures; consequently they first paralyse inhibition, and so lead to an increased expenditure of nervous energy, and so are stimulants, but later, and especially after large doses, they paralyse also the structures from which spring nervous energy, and so paralyse. To take an example, we have endeavoured to show that strychnia increases the expenditure of force in the cord by paralysing those structures which control the amount and limit the area of the cord affected; but strychnia has also a weak affinity for the elements which produce nervous energy in the cord, and hence after a large dose these elements also become paralysed, and hence we have first a stimulating action and next a paralytic.

Take another case: digitalis in small doses stimulates the inhibitory apparatus of the heart, but in large doses paralyses it. We assume that digitalis has a far greater affinity for those parts of the inhibitory apparatus which control the conduction of impressions through the vagi nerves, or the evolution of energy from the intra-cardiac inhibitory apparatus, and a weaker one for the nerve elements or the cell elements which produce the inhibitory energy. Owing to its greater affinity for those structures controlling the evolution of energy, digitalis at first "stimulates" the inhibitory apparatus, and so slows the heart, but a large dose is sufficient to paralyse the structures which supply the inhibitory energy and destroy inhibition, and then quicken the heart.

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ON THE ANTAGONISMS OF ACONITIA ON THE FROG'S HEART.

By SIDNEY RINGER, M.D., *Professor of Medicine in University College, London.*

I. On the Action of Aconitia itself on the Heart of the Frog.

WHEN aconitia[1] is injected under the skin of a frog in doses from $\frac{1}{100}$th to $\frac{1}{10}$th grain, loss of sensation, as indicated by the inability of stimuli to call forth reflex actions, comes on in a few minutes, and all voluntary movements are lost in about half an hour. If the heart be then laid bare in the usual way, it will be found, according to the dose, to be either beating more slowly and feebly than usual or to be in complete standstill with the cavities widely distended. The beats, which after the small doses may continue for one or two hours, are not only slower and feebler but also present certain marked features. The auricles beat much more rapidly than the ventricle, so that two or more beats of the former occur to one of the latter. Moreover the contractions of the ventricular walls are irregular and incoordinate; one part only of the ventricle will often contract by itself and drive the blood to another part causing this to become distended and pouched; the pouched portion will, in many cases, subsequently take up the contraction, the previously contracted portion becoming pouched in turn. Thus a wave, so to speak, of contraction and pouch-like distention may pass over the ventricle rendering the whole beat highly irregular. Aschscharumow (*Archiv f. Anat. u. Physiol.*, 1866, p. 255) who noticed this remarkable effect of aconitia believed it to occur in the final stages only of poisoning; but I have seen it even in quite early stages.

After even these irregular incoordinate contractions have ceased, and the ventricle has come to a complete standstill, no spontaneous contractions at all appearing, a beat of the ventricle may be evoked by direct mechanical stimulation; and the beat thus artificially produced exhibits in many cases the same features of pouching through partial incoordinate contraction.

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[1] I used Hopkins and William's aconitia dissolved by the addition of dilute sulphuric acid, two minims to each grain.

It is evident that aconitia acts for some reason more powerfully on the ventricle than on the auricles, and that it affects the machinery (automatic ganglia) which carry out spontaneous beats earlier than the machinery (reflex ganglia, muscular substance) by means of which artificial stimulation gives rise to a beat. Further, it has a peculiar effect either on the muscular substance or the ganglia, leading to the beat whether spontaneous or induced having the special characters described above.

I may add that the fact of the heart continuing to beat for a long time after general paralysis has set in is a clear proof of the view which I have elsewhere urged that the general paralysis cannot possibly be merely the result of failure of the heart's beat.

II. The Antagonism between Aconitia and Atropia.

The following five experiments will serve to illustrate the relative actions of the two drugs.

Sept. 15. I injected a tenth of a grain of aconitia into a frog weighing 39 grammes. In three minutes sensation and reflex action were abolished, and voluntary movement in twenty-four minutes. I then exposed the heart and found the ventricle motionless and widely distended, but the auricles contracted well at 22 per minute. Twenty-seven minutes after poisoning each mechanical stimulus excited a weak incoordinate ventricular contraction. Thirty-two minutes after the poison mechanical stimulation failed to excite any ventricular contraction, but the auricles beat spontaneously 22 good contractions per minute. Thirty-five minutes after poisoning I applied to the heart a small quantity of a 2 per cent. solution of atropia, and in six minutes each mechanical stimulus excited a good ventricular contraction. The auricles continued to beat spontaneously. I took observations every five minutes, and the heart continued in exactly the same state till twenty-nine minutes after the application of atropia, when *spontaneous ventricular* contractions commenced. In thirty-one minutes the ventricle beat fairly well at 8 per minute, and in thirty-seven minutes 14; in forty-three minutes, 15 fair beats per minute. Two hours and a quarter after the application of atropia the ventricle beat well spontaneously about one beat per minute, and each mechanical stimulus excited an effective ventricular contraction. The auricles at this time beat spontaneously 20 good beats per minute.

Sept. 16. I injected $\frac{1}{20}$ gr. of aconitia into a frog weighing 40 grammes. Reflex action disappeared in seven minutes and voluntary power in twenty-one. Twenty-seven minutes after the poisoning I exposed the heart. The ventricle was motionless, widely extended, and did not contract on mechanical stimulation. The auricles beat spontaneously but slowly and somewhat feebly. In twenty-eight minutes I applied a small quantity of a 2 per cent. solution of atropia. In one minute the auricular action improved, beating 18 per minute, but the ventricle remained motionless and refused to contract under a mechanical stimulus. In six minutes the auricles beat well at 22, and mechanical stimulation produced a distinct though rather weak ventricular contraction. In nine minutes spontaneous ventricular contraction begun, 12 per minute, irregular in character but fairly strong. To each beat of the ventricles the auricles beat twice, but in thirteen minutes the auricles and ventricle beat alike in frequency, 14 good regular spontaneous beats per minute. In eighteen minutes the beats rose to 24 per minute. Ninety-three minutes later the heart beat fairy well and regularly at 14 per minute.

Sept. 16. I injected a frog weighing 47 grammes with $\frac{1}{20}$ grain of aconitia. Reflex action ceased in two and voluntary power in twenty-two minutes. Then I exposed the heart and found both auricles and ventricles beating, the ventricle contracting badly and incoordinately. During the following thirty-two minutes the ventricular contractions gradually fell to only one or two beats per minute, the auricles however continuing to beat from 24 to 28. Then on applying a little of a 2 per cent. solution of sulphate of atropia, in six minutes the ventricular contraction beating five in the minute became almost coordinate. In eight minutes they rose to 14; in ten minutes to 20, and became almost coordinate. During the following forty minutes, *i.e.* till fifty minutes after the application of atropia, the heart continued beating fairly well coordinately at 18 to 19 per minute. Sixteen hours after the poisoning the heart beat fairly well at 12, and eighteen hours later 10 weak beats per minute. Twenty-one hours afterwards the heart stopped widely distended, but a mechanical stimulus excited a weak contraction.

Sept. 16. I injected a frog weighing 47 grammes with $\frac{1}{20}$ grain of aconitia. Reflex action ceased in five, and voluntary movement in seventeen minutes. Eighteen minutes after the poisoning I exposed the heart and found both auricles and ventricles beating at 14 per minute, the contractions of the ventricle being very incoordinate. During the ensuing sixty-two minutes the contraction varied from 14 to 16 per minute, the action of the ventricle continuing very incoordinate. I then applied a small quantity of a 2 per cent. solution of sulphate of atropia. The pulsations rose to 20 and the contractions of the ventricle became a little stronger and strictly coordinate.

Sept. 17. I injected $\frac{1}{10}$ grain of aconitia into a frog weighing 35 grammes. Reflex action ceased in three and voluntary movement in ten minutes. Twelve minutes after poisoning I exposed the heart. The ventricle contracted strongly but very incoordinately at 2 per minute, whilst the auricles contracted well at 28 per minute. Six minutes later spontaneous contractions ceased in the ventricle, but during the following fourteen minutes (that is till thirty-two minutes after the poisoning) a mechanical stimulus excited a strong but incoordinate ventricular contraction. The auricles all this while contracted well at 24 to 28 per minute. I then applied a small quantity of a 2 per cent. solution of sulphate of atropia. In six minutes I found that a mechanical stimulus more easily induced a ventricular contraction. The heart continued in the same state till seventeen minutes after dosing with atropia, when spontaneous ventricular contractions begun strong from the first though very incoordinate. Thirty minutes from the application of atropia the ventricle beat strongly but rather incoordinately at 14 per minute, and the auricles regularly at 28 per minute. Two hours after dosing with atropia the ventricular contractions had fallen to 2 strong incoordinate contractions per minute and the auricular to 10. I then applied the atropia, and during the ensuing twenty minutes the ventricular contractions rose to 8 and became strong and almost coordinated. Five hours after the second application of atropia the ventricle contracted 7 strong coordinated beats per minute.

The results of these five experiments may be summarized as follows:

First case. The aconitia completely paralysed the ventricle so that even a mechanical stimulus failed to excite a contraction. Six minutes after applying atropia, a mechanical stimulus produced a contraction; in twenty-nine minutes spontaneous ventricular contractions begun and rose to 14 fair beats per minute. The spontaneous contractions persisted two hours and a quarter.

Second case. The aconitia completely paralysed the ventricle, so that even a mechanical stimulus failed to excite a contraction. Six minutes after the application of atropia, a mechanical stimulus excited a ventricular contraction; in nine minutes spontaneous ventricular contractions begun and rose to 20 good coordinate beats, continuing more than ninety minutes.

Third case. The aconitia almost arrested the ventricle which beat only once per minute. Six minutes after dosing with atropia the contractions improved and rose to 20 fair coordinate beats.

Fourth case. The aconitia weakened the ventricular contractions and made them very incoordinate. Atropia increased the frequency of the beats by four and made them stronger and coordinate.

Fifth case. Aconitia arrested the spontaneous ventricular contractions, but a mechanical stimulus excited an incoordinate contraction. Seventeen minutes after applying atropia spontaneous ventricular contractions begun and in fourteen minutes they became coordinate.

In every instance atropia effectually antagonized the action of aconitia. Where the ventricle was completely paralysed, atropia restored the pulsations; where the pulsations were only slowed, weakened, and incoordinate, atropia strengthened, accelerated, and coordinated them. The suspended ventricular contractions returned in the following way: first, a mechanical stimulus excited an incoordinate but otherwise fairly good contraction, then some minutes later, spontaneous ventricular contractions began, at first incoordinate, but which became coordinate.

On the view that the absence of response to mechanical stimulation is due to a loss of power of the muscular substance we may conclude that the antagonism of atropia and aconitia manifests itself in the muscular substance of the heart before it is brought to bear on the (ganglionic) nervous machinery which carry out the spontaneous contractions. In the language of the hypothesis put forward on a previous occasion (this *Journal*, Vol. II. 1879, p. 235)[1], atropia has a stronger affinity for the cardiac tissues than has aconitia, the latter consequently being replaced by the former, first in the muscular and subsequently in the nervous ganglionic tissue.

It may be remarked that the antagonistic action of atropia, though unequivocal, is not absolutely complete. Atropia does not restore the aconitized heart to a perfectly normal condition. The heart's beats, though they may be restored to perfect coordination, remain slower and especially weaker than those of a natural heart. Moreover the antagonism may in certain cases be absent or manifested to a slight degree only, especially where the action of aconitia is very profound. Thus in the case of three frogs poisoned with $\frac{1}{10}$ or $\frac{1}{20}$ aconitia, in two of which even the auricle had ceased to beat, the recuperative effects of a 2 p.c. solution of atropia were very slight and confined entirely to the auricle, no beat spontaneous or artificial could be observed in the ventricle.

III. The action of Aconitia on the Muscarized Heart, and of Muscarin on the Aconitized Heart.

When the heart is brought to a standstill[2], either rapidly by the direct application of a large dose, or more slowly by repeated applications of smaller doses of muscarin, no visible effect is produced by the subsequent application of aconitia; no contractions make their appearance, the heart remaining motionless and quiescent. This is what one might expect, since the effect of both muscarin and aconitia is to weaken and arrest the heart's beats. If the application of the aconitia be followed by that of atropia, the beats are resumed, being at first incoordinate, as after aconitia poisoning, but subsequently regular and strong. From this we might infer that the aconitia does really replace the muscarin, so that the standstill which is continued after the aconitia has been given is different from that previously due to the action of the muscarin, though we are unable to perceive these differences until the quiescence of the heart is broken by the influence of atropin.

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[1] It is only just to Mr Langley that I should take this opportunity of expressing my regret, that it had escaped my notice that in a paper in this *Journal* (Vol. I. p. 367), he had put forward an hypothesis concerning the action of drugs of a wholly similar nature; I may at the same time be allowed to congratulate myself that so acute an observer has been led to adopt and the first to formulate a view at which I had myself independently arrived.

[2] As Langley has already noticed, the direct application of the small fragment of muscarin-extract to one portion of the ventricular walls leads at first to a local effect confined to that portion. The contractions in this portion become feeble, and soon the part ceasing to contract at all bulges out in a pouch during the contraction of the rest of the ventricle. Subsequently this local effect spreads over the rest of the cardiac substance until the whole heart becomes quiescent.

When muscarin is given to a heart brought to a standstill by aconitia there is in like manner no visible effect; the heart still remains motionless. As in the previous case the beats are restored by atropin, and here also the dominant influence of the aconitia makes itself felt by the lack of coordination in the earlier beats of the restoration.

IV. The relations of Aconitia and Pilocarpine.

The following experiment will serve to illustrate the relation of these two drugs in reference to the heart.

Sept. 19. I injected a frog weighing 35 grammes with $\frac{1}{20}$ grain of aconitia. In twelve minutes, when paralysis became complete, I exposed the heart, opened the pericardial sac and found the ventricle and auricles contracting well, the ventricle once and the auricles 20 times per minute, then I applied a 2 p. c. solution of nitrate of pilocarpine. In three minutes the ventricle completely stopped beating and the auricles almost stopped. In seven minutes I applied a little of a 10 p. c. solution of nitrate of pilocarpine, and fifteen minutes later, whilst the ventricle remained motionless, the auricles beat well 26 per minute. In thirty-four minutes the heart remained in the same condition and mechanical stimulation failed to provoke a ventricular contraction. I then applied a 2 p. c. solution of sulphate of atropia. In two minutes the auricles beat well at 48 per minute. In eleven minutes mechanical stimulation excited a ventricular contraction. In fourteen minutes spontaneous ventricular contractions began. In eighteen minutes the ventricle contracted pretty well, but very incoordinately, 14 times a minute. Thirty minutes after the application of the atropia the ventricle contracted, coordinately and pretty well, 16 per minute.

From this and the similar results of other experiments it would appear that pilocarpine will not antagonize aconitia in the sense of restoring the beats of a heart brought to a standstill by that drug. But as in the case of muscarin and aconitia, so also in the case of pilocarpine and aconitia, restoration by atropia brings out the (latent) special effect of the aconitia as shown by the, at first, incoordinate contractions.

Thus while atropia and pilocarpine both antagonize muscarin, atropia only will antagonize aconitia in the sense of restoring the heart's beat.

The conclusions arrived at in this paper may be summarized as follows:

1. Aconitia slows and weakens the heart and incoordinates the ventricular contraction, this incoordination occurring before the heart's contractions are greatly weakened.
2. Sometimes the contractions though greatly slowed and very incoordinate continue fairly strong till the heart stops.
3. Aconitia acts on the ventricle far more rapidly and powerfully than the auricles.
4. Aconitia has a more powerful action on the cerebro-spinal centres than on the nervous structure of the heart, for after complete paralysis the heart often continues to contract well for a considerable time.
5. The general depression from a poisonous dose of aconitia is partly and perhaps chiefly due to the direct action of the drug on the nervous centres, rather than to the weakening the circulation, consequent on the failure of the heart.
6. Atropia antagonizes the action of aconitia on the heart. It restores the contractions in a heart arrested by aconitia, and strengthens, accelerates, and coordinates the heart simply weakened, slowed, and incoordinated by aconitia.
7. Aconitia does not antagonize the action of muscarin, nor can muscarin antagonize the action of aconitia on the heart.
8. Atropia antagonizes the combined effects of aconitia and muscarin.
9. Pilocarpine does not antagonize the action of aconitia on the heart.
10. Atropia antagonizes the combined effects of aconitia and pilocarpine.
11. I have suggested that the antagonisms are due to chemical displacement. Atropia antagonizes muscarin, pilocarpine, and aconitia because it has a stronger affinity for the muscular and nervous structures of the heart than these substances, and displaces them, replacing their effect by its own.

CONCERNING THE INFLUENCE OF SEASON AND OF TEMPERATURE ON THE ACTION AND ON THE ANTAGONISMS OF DRUGS. By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.*

I. The Influence of Season on the Antagonism of Pilocarpine and Muscarin.

I HAVE elsewhere shown that pilocarpine[1] antagonizes the action of extract of muscaria on the frog's heart, and atropia the action of aconitia[2]. On reverting to this subject at another season of the year, I was astonished to find that these antagonisms were far less marked, and, in many instances, were indeed absent altogether. With the assistance of Dr. Murrell and Mr. Morshead I have re-investigated the subject, only to find that these antagonisms are largely influenced by temperature.

I first noticed in October that the antagonism between pilocarpine and extract of muscaria was much less marked than in my previous experiments in July and August. In my winter experiments I found that the degree of antagonism varied very considerably. The antagonism was much more marked in frogs kept in a warm room than in frogs kept in the cold cellars, and this led me to attribute the difference in the amount of antagonism to difference of temperature, and the following experiments are designed to test the correctness of this supposition.

During each month, from October to July inclusive, I made about a dozen experiments with pilocarpine and extract of muscaria on frogs kept in the college cellars, where the temperature is much the same as that of the external air. I find that the amount of antagonism depends largely on the temperature.

In January I kept some frogs in a warm chamber at 60° Fah., and with these animals I found the antagonism nearly as perfect as in my previous experiments made in summer.

I used a 1 in 5 solution of extract of muscaria, and a 2 per cent.

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[1] *Journal of Physiology*, Vol. I.

[2] *Journal of Physiology*, Vol. II.

solution of nitrate of pilocarpine. Both gave an acid reaction, the nitrate of pilocarpine being the more acid.

I destroyed the brain, and then divided the thorax, and cut through the pericardium, and applied the extract of muscaria to the heart, and when it had stopped I applied the pilocarpine solution.

In Table I. I give the average results of experiments made from October to June inclusive, on the hearts of frogs kept at the natural temperature of the season.

Antagonism on Heart of Frogs kept in the Cellars.

Month.	Number of experiments.	After application of pilocarpine the beats increased on an average	Heart continued beating on an average	After the application of pilocarpine the beat became
October	6	12 beats	107 minutes	Good in two. Fair in one. Very weak in four.
November	9	12 beats	46 minutes	Good in four. Very weak in two. No antagonism in three.
December	11	3 beats	30 minutes	Good in one. Weak in five. No antagonism in five.
January	10	7 beats	86 minutes	Good in five. Weak in two. No antagonism in three.
February	7	4 beats	24 minutes	Weak in three. No antagonism in four.
March	10	3 beats	35 minutes	Good in three. Weak in one. No antagonism in six.
April	11	12 beats	4 hours	Good in eight. Weak in three.
May	12	23 beats	9 hours	Good in ten. Weak in two.
June	9	40 beats	20 hours	Good in all.

This table shows the marked influence of the season on the antagonism of pilocarpine for extract of muscaria. This is shown in the number of beats restored to the heart after the application of the pilocarpine, and also by the long time the heart continued beating, and by the number of cases in which the antagonism occurred. In the colder months, as October to March inclusive, the antagonism was slight and often absent, whilst in the warmer months the antagonism was strongly marked, and in proportion to the height of the temperature.

I may here repeat that in different experiments in each month the amount of antagonism often varied considerably, and this difference was always associated with change of temperature. After three or four warmer days the antagonism became more marked.

In December and January I made fourteen observations on frogs kept, on an average, five days in a Page's warm chamber, at 60° Fah., and in these the antagonism was well marked, as the following table of the averages show.

After the application of pilocarpine the beats increased on an average	The pulsations continued on an average	The pulsations became
27	25 hours	Good in every instance.

These experiments on frogs kept several days at about 60° Fah. conclusively prove that temperature influences the antagonism between pilocarpine and extract of muscaria, and that the antagonism is well marked only in frogs kept at about 60° Fah.

The recovery in the frequency and strength of the heart's beats, after the application of pilocarpine, is not a simple recovery from the effects of the extract of muscaria, but is due to the antagonizing effects of pilocarpine; for in thirty test experiments made in October, November, and December, with extract of muscaria alone, in two only did the heart improve; in the rest the heart grew progressively weaker, and in a short time stopped.

In order that the antagonism should be well marked the frogs must be kept in a warm temperature several days. I noticed this in frogs kept in the laboratory. If kept there for one day only, no more than a slight or no antagonism occurred at all; but if kept there several days the antagonism became much more decided.

In January I destroyed the brains of six frogs just brought from the cellars and exposed their hearts, and then placed the animals in a warm chamber at 75° Fah., and after fifteen minutes I tested the antagonism of pilocarpine and extract of muscaria. After the application of pilocarpine the beats rose on an average 3, and the pulsations continued on an average 11 minutes. In three experiments the beats were very weak, and in three there was no antagonism.

We may conclude, therefore, that if the temperature of the heart is quickly raised to the temperature of summer, the antagonism remains so slight that it is almost nil, and I find that it requires several days at a warm temperature to establish the antagonism.

The antagonism of pilocarpine and extract of muscaria is most marked on the auricles, for the action of muscaria is much more marked on the auricles than on the ventricle. On the application of extract of muscaria the auricles speedily become motionless, whilst the ventricle may for several hours continue beating. Yet almost at once the pilocarpine restores good contractions to the auricles.

During the period of sexual activity the antagonism I noticed was but slight.

The difference in the amount of antagonism at different seasons of the year, and dependent on differences in temperature, shows that the affinities of the protoplasmic molecule are modified by temperature, and implies that the chemical composition of the molecule undergoes some change from the effects of temperature.

CONCLUSIONS.

1. The antagonism of pilocarpine and extract of muscaria on the frog's heart varies in different months.

2. In the summer months pilocarpine always strongly antagonizes extract of muscaria; but in the winter months there is often no antagonism, and even when it occurs, it is generally slight.

3. This difference is due to temperature, for if in the winter months frogs are kept for several days at 60° Fah. the antagonism becomes well marked, and indeed as strong as in the summer months.

4. This seasonal difference in the antagonism of these two agents suggests that in summer and winter respectively there is a difference in the composition of the protoplasmic molecules of the cardiac structures of the frog's heart.

II. The Influence of Season on the Antagonism of Atropia and Aconitia.

In Vol. III., No. 1, of the *Journal of Physiology*, I have recorded some experiments showing the antagonism of atropia and aconitia in the frog's heart. These experiments were made in September. Atropia, I found, restores the contractions in a heart arrested by aconitia, and strengthens, accelerates, and co-ordinates the heart simply weakened, slowed, and in-coordinated by aconitia.

On repeating these experiments in December, January, and March, I found the antagonism either absent or very slight.

I injected $\frac{1}{10}$ grain of aconitia under the back of frogs weighing from 33 to 76 grammes, and when the animal was nearly paralyzed I destroyed the brain, then exposed the heart, divided the pericardium, and applied a 2 per cent. solution of sulphate of atropia. The aconitia was dissolved by the aid of a small quantity of sulphuric acid, and the solution consequently had a slight acid reaction.

Before recording my results, I may first state that aconitia exercises a far more powerful effect on the ventricle than on the auricle. Indeed, in some cases, the auricles beat well even when the ventricle was completely paralyzed, so that mechanical stimulation failed to excite it to contraction. It is needless to describe the experiments in full, so I shall record only the results.

In December and January I made eleven observations. In eight of these there was no antagonism in respect of the ventricle; in the remaining three was a slight ventricular antagonism. In every instance the atropia markedly antagonized the action of aconitia on the auricle and restored good contraction.

In March I made six experiments. In four the atropia did not antagonize the action of aconitia on the ventricle; in one good, and in another feeble contractions returned to the ventricle; but in all the experiments good contractions returned to the auricles.

I next made, in December, a very few experiments regarding the influence of temperature on the antagonism, and although these experiments are too few to be decisive, they are perhaps worth recording.

In the case of a frog kept at 60° Fah. in the warm chamber for 15 hours, no antagonism occurred in the ventricle. The auricles beat well on the application of atropia.

I kept two frogs at 60° Fah. for 46 hours. In one a moderate amount of antagonism occurred; in the second no ventricular antagonism took place. In one there was marked auricular antagonism; in the other case the auricles beat well before the application of atropia.

These experiments seem to make it probable that 46 hours is insufficient time to change the condition of the heart and to restore the antagonism of atropia and aconitia.

It appears, then, as in the case of pilocarpine and extract of muscaria, that the antagonism of atropia for aconitia varies greatly at different times of the year; and I conclude that the amount of antagonism is closely related to temperature: that the antagonism is well marked in the warm months, and is absent in the ventricle in the cold months.

Pantelejeff[1] finds that the antagonism of atropine for quinine on the frogs' hearts is affected by the time of year. In summer frogs, quinine arrests the heart in diastole, and atropia causes the heart at once to resume its pulsations. In winter frogs, quinine arrests the heart much more slowly, whilst atropine instead of obviating actually increases this arrest.

III. The Influence of Temperature and Season on the Action of Atropia on the Heart.

The action of atropia on the heart is different in the cold months as compared with its action during the warm months. I have tested the action of atropia in the winter months by the application of the solution to the heart *in situ*, exposed by opening the thorax. I have also used Roy's tonometer. I made my observations in January, February, and March, and though I have careful notes of eleven experiments only, I performed many more. I used a 2 per cent. solution of sulphate of atropia and applied it freely to the heart. The solution was never colder than the frog's heart, but may have been sometimes a little warmer, as whilst the frogs were kept in the cellar the solution was kept in the physiological laboratory where the experiments were performed.

In all these experiments the heart was much slowed, though each cardiac contraction was much strengthened. The diastolic dilation of the ventricle was decidedly increased, and the ventricular contraction grew much stronger and prolonged, the ventricle becoming very small and pale. Immediately on the completion of the systole the ventricle, even before the auricular contraction, became widely distended with blood.

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[1] *Lancet*, July 31, 1880, p. 176.

The hearts beat on an average 35 per minute before the application of atropia, and afterwards fell on an average to 19·5. Thus the atropia reduced the number of beats to nearly one half.

Whilst each beat became stronger and propelled more blood, it was evident that there was a decided diminution of the amount of heart-work done in a given time. The great reduction of the frequency of the heart's beats, in fact to nearly half, more than compensates for the increased force of each systole. Therefore, though this agent strengthens each contraction and increases the quantity of blood it propels, yet since it diminishes the total amount of work done, atropia must be considered a weakener of the heart.

Tracings by means of a lever placed on the heart demonstrate that both the systole and the diastole are greatly prolonged, especially the diastole. The proportionately greater increase in the diastole over the increase in the systole clearly shows that atropia must diminish the amount of work done in a given time.

Experiments with Roy's tonometer likewise fully confirm my previous statements, and show that both diastole and systole are much prolonged, the diastole largely in excess of the systole.

In summer atropia affects the heart differently. Soon after the topical application the heart becomes slowed to about half its previous rate, with very prolonged diastole, and during this period the heart lies empty and flaccid. The auricular contraction forces but little blood into the ventricle, which consequently becomes very imperfectly distended, and the ventricle on contraction does not become very pale. The heart is indeed not only, it is evident, considerably slowed, but the circulation is greatly retarded, and to me it seems that each ventricular contraction is weaker.

Next I undertook some observations to ascertain whether the effect of atropia, so different in the summer from the effect in winter, is due to temperature.

I exposed the hearts of four brainless frogs, and placed them in the warm chamber. Having kept the frogs in the warm chamber (about 70° Fah.) long enough to accelerate the hearts from about 30 to about 60 beats per minute, I freely applied a 2 per cent. solution of sulphate of atropia of about the same temperature as that of the animal's tissues. The atropia I found affected these hearts so treated in the same way as it affects the hearts of summer frogs. The difference, therefore, in the action of atropia in the winter contrasted with its action in the summer, is largely, if not entirely, due to temperature.

All this difference, however, as regards the summer (warm) heart, cannot be attributed to the effect of atropia on the ventricle, but must be due to its influence either on the auricles or on the blood-vessels. The ventricle of the summer heart when treated by atropia becomes, during diastole, only imperfectly distended with blood. Roy[1] has shown that the size of the ventricle, or in other words, the amount of blood it receives, depends on the amount propelled into it by the auricles. Therefore the undistended ventricle, after the application of atropia, must be due either to feeble contractions of the auricles or to their receiving but little blood to propel; and I think the latter alternative affords the true explanation.

CONCLUSIONS.

1. Atropia in winter affects the heart in a very similar way to cold. Both diminish the number of beats, prolong and strengthen the systole, prolong the diastole, and increase the diastolic dilation of the ventricle. The diastole is prolonged much more than the systole.
2. In summer atropia lessens the amount of blood going to the ventricle during diastole, for during the auricular contraction the ventricle still remains only partially filled, and I think the ventricular contraction is weakened.
3. In both winter and summer atropia lessens the amount of heart work; for even in winter, when each ventricular contraction is stronger, so as to propel more blood at each stroke, the great reduction in the number of beats more than compensates for the increased force of each contraction.

IV. The Influence of Temperature and Season on the Action of Muscarin on the Heart.

The effect on the frog's heart of extract of muscaria in winter differs from its effects in summer. In the winter months the extract or cold affect the heart in almost exactly the same way, the correspondence being greater than that between cold and atropia: for atropia never slows the heart to the extent that great cold does, whilst extract of muscaria slows the heart equally with cold at or even rather below freezing-point.

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[1] *Journal of Physiology*, Vol. I., p. 452.

In these experiments—made in January, February, and March—I used a 1 in 5 solution of extract of amanita muscaria.

If topically applied to the heart in the winter months extract of muscaria greatly slows and increases the strength of the ventricular contraction, affecting the ventricle, as I have said, just like cold or like atropia in the winter months. The diastolic dilation is decidedly increased and the systolic contraction grows much stronger, the ventricle becoming smaller and paler, whilst the duration of the systole is prolonged. The contractions in many cases fell to two in the minute, and yet they were stronger than before the addition of the extract of muscaria. This increased diastolic dilation and increased systolic contraction often continues till the heart stops; in other cases the strengthened contractions persist a variable time, then the ventricular contractions grow weaker and weaker, till at last, reduced to a mere flicker, they cease in wide diastole. This effect on the ventricular contraction we observed in our January experiments, not only in frogs brought from the cellars but in frogs kept in a warm chamber for four to six days; but the primary strengthening of each contraction persisted much longer in the cold than in the warm frogs, sometimes continuing in the cellar frogs for more than half an hour.

The effect on the auricles is very different, for in every instance the contractions at once grew weaker and soon ceased, so that we had a heart with paralyzed auricles, but with ventricle freely dilated and very strongly contracted.

It appears then that the different effect of extract of muscaria in the winter compared with the summer months is limited to the ventricles.

Roy has shown that the degree of ventricular dilation depends on the amount of blood propelled by the auricular contraction and the venous pressure. In our experiments the ventricular distension was due to the blood sent into the ventricles chiefly from the veins. For we have seen that immediately on the application of extract of muscaria the auricles grew weak and soon ceased beating, yet the ventricles still became widely distended during each diastole, so that at first the greater part, and finally the whole, of the distension was due to the veins.

The share the veins played in producing the ventricular dilation became manifest even whilst the auricles continued beating. The heart became greatly slowed, and at the moment of completion of the systole the ventricle at first rapidly dilated and became filled with blood, but towards the end the dilation became relatively much slower, and then the auricles contracted, increasing in a slight degree only the ventricular distension. The increased diastolic distension of both the auricles and the ventricle is, I think, susceptible of explanation. The extract of muscaria reduces greatly the frequency of the heart's contractions, and thus gives ample time for the arteries, both by virtue of their elasticity and of their muscular contraction, to empty themselves, to distend the veins, and to heighten venous tension. This heightened venous tension, aided by the prolonged diastolic pause, forces a larger and unusual quantity of blood into both auricles and ventricle, and causes in both unwonted distension. The auricles being weakened do not empty themselves with each contraction, and soon become motionless; but the ventricle being strengthened the whole of its blood is strongly propelled into the aorta.

After the application of extract of muscaria it is true that each ventricular contraction decidedly gains in strength, and owing to the increased diastolic dilation more blood is propelled with each ventricular contraction; still, as in the experiments with cold or atropia, the heart does a far less total amount of work. The application of extract of muscaria solution may, for instance, reduce the beats to 6 or even to 2 in the minute, and though each beat, after the application of the extract, propels more blood with greater force than before, yet there must be a marked falling off in the total amount of work done in a given time.

To eliminate the muscular contractility of the arteries and to observe how the heart comports itself when treated with extract of muscaria, I destroyed not only the brain but likewise the spinal cord, and then having exposed the heart, I applied the solution. To my surprise the muscaria instead of strengthening the ventricular contraction at once greatly weakened it, and in a few minutes arrested it. Muscaria, in fact, acted on the heart in all respects as in the summer months.

In numerous tracings of the ventricle with the groove attached taken with Roy's tonometer, I never found extract of muscaria to produce any primary strengthening, but the curve at once grew lower, sharper, and shorter.

Fig 1, A.

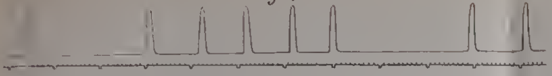


Fig. 1, B.

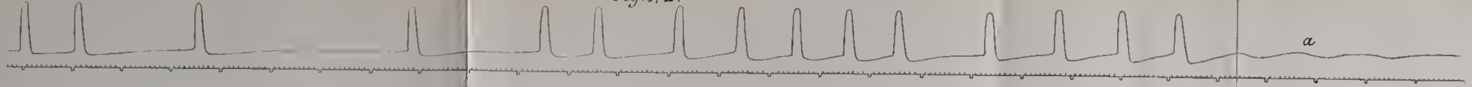


Fig. 1, C.

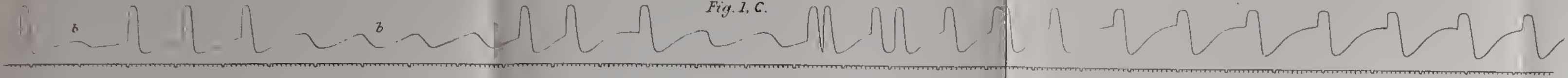


Fig. 1, D.

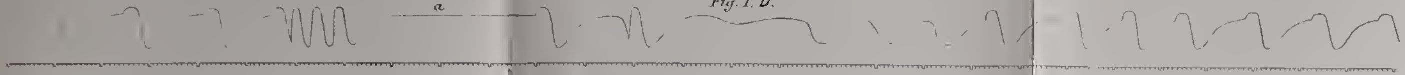


Fig. 1, E.

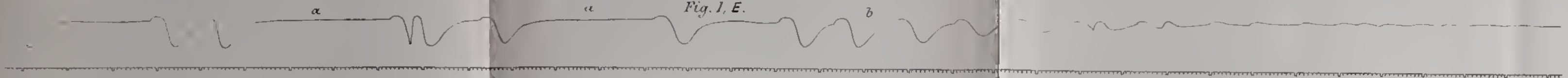
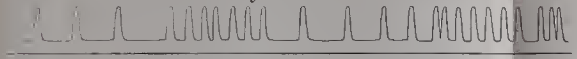


Fig 2 A.



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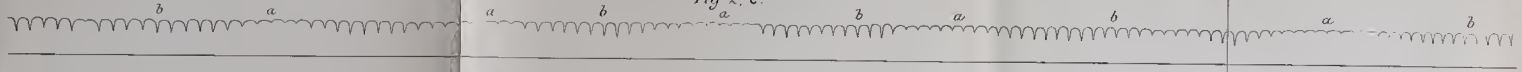

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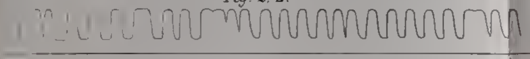

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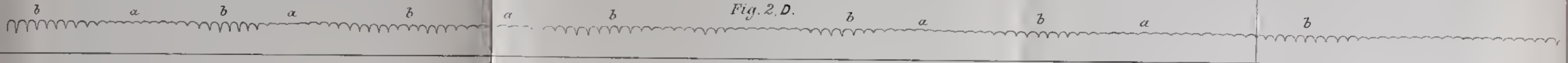

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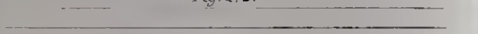


Fig. 3, A.

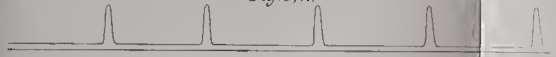


Fig. 3. B.

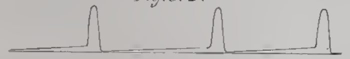


Fig 3, C.

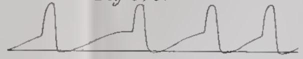


Fig. 3, D.

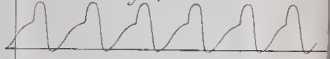


Fig. 3, E.

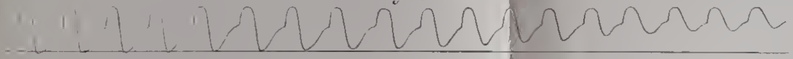


Fig. 3. F.

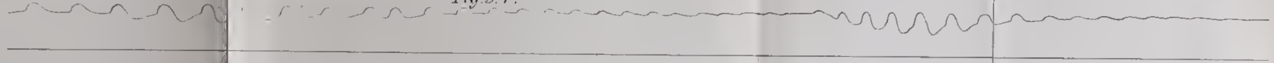


Fig 4. A.

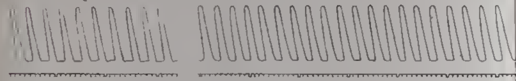


Fig 4 B.

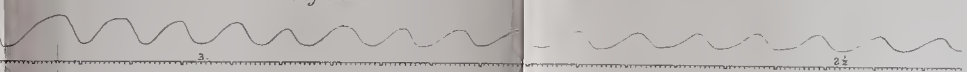


Fig 5. A.

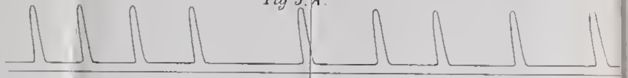


Fig. 4. C.

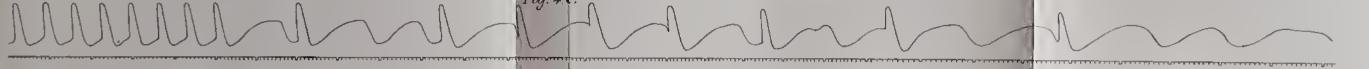
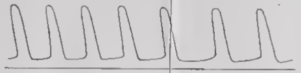


Fig 5, B.



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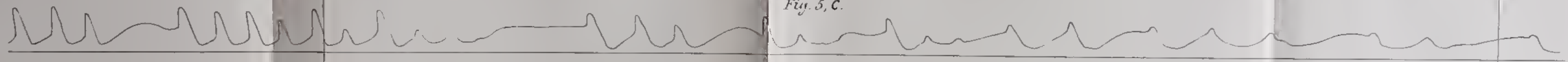
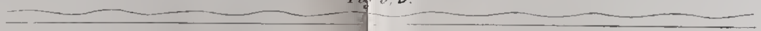


Fig 5, D.



P. Huth, Lith. Edin.

REGARDING THE ACTION OF HYDRATE OF SODA, HYDRATE OF AMMONIA, AND HYDRATE OF POTASH ON THE VENTRICLE OF THE FROG'S HEART.

By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.* Pl. XII., XIII.

In No. 1, Vol. III., of the JOURNAL OF PHYSIOLOGY Dr. Gaskell records some experiments regarding the respective action of antiarin, hydrate of soda, muscarin, and digitalin on the ventricle of the frog's heart.

In this paper I record some experiments on the action of soda, ammonia, and potash on the same structure. In most respects my experiments with soda hydrate agree with those of Dr. Gaskell; in some respects, however, my tracings differ from his tracings. These experiments were made with Roy's tonometer. I tied the ventricle to the cannula by a ligature passed round the auriculo-ventricular groove, so that I experimented only with the ventricle.

I used dried bullock's blood dissolved in water so as to represent normal blood; to this solution I added saline solution in the proportion of one part of blood to two of saline. Of this mixture I employed three ounces in each experiment.

These experiments were made in June.

The tracings run from left to right.

I find that soda, ammonia, and potash affect the ventricle in much the same way. There are, however, differences in their action. I shall first describe the characters in which they all agree, and then point out in what respect the action of potash differs from the action of soda and ammonia.

Ammonia.

The general effect of all these substances is indicated in Fig. 1, Pl. XII., which shows the modifications produced by ammonia. The solution added to the blood contained 1 per cent. of strong liquor ammonia[1].

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[1] I added a few drops of the 1 per cent. solution (soda or ammonia or potash) from time to time to the three ounces of blood. The quantity of the salt used is given with the explanation of plates.

Ammonia (see B) prolongs each contraction, and the summit of each rise becomes rounder; at the same time, during each diastole, the trace rises a little, but after each rhythmic contraction falls again to its old level as regards the base-line. If the rhythmic contractions cease for a time, the trace then rises as before and becomes undulating (see *a*). An inspection of the heart explains this undulating line. After the last rhythmic contraction the ventricle slowly lessens in size, and then rather more suddenly dilates, first at the apex, and then the dilatation extends to the base, or the dilatation begins at the base and extends to the apex; then the ventricle again slowly retracts and again dilates, and so on. After a larger dose of ammonia (see C, D and E) the rise in the trace during diastole becomes still more evident, and may, indeed, become so great that the ventricle shrinks almost to the dimensions it assumes at the termination of a rhythmic contraction. During a long diastolic pause the undulations in the trace become much more marked. Under the influence of a still larger dose the undulations disappear; the trace slowly rises after the dilatation, following a rhythmic contraction, and then continues at the same height during the remainder of the diastolic pause. After each rhythmic contraction the ventricle dilates to a far greater degree than immediately before the contraction. Under larger and larger doses the diastolic dilatation after each rhythmic contraction becomes less and less, until finally the diastolic dilatation (see E) fades away, and the trace becomes a slightly wavy line.

Fig. 2 shows the production of some further effects when ammonia is added in larger doses. In this trace there are no long diastolic pauses. This trace shows that with frequent ventricle beats, there is no diastolic contraction of the ventricle, as seen in Fig. 1, there being no time allowed for this diastolic retraction. But the effect of ammonia is shown in a lessened expansion of the ventricle in each diastolic dilatation.

After a large dose of ammonia (see C and D), the amplitude of the trace varies from time to time; at one time very small, it then grows gradually larger and again diminishes, and so on: the increase in the size of the trace is due entirely to an increase in the diastolic dilatation, this varying from time to time.

These variations in the size of the rhythmic contractions correspond to the undulations seen in Fig. 1 (B, a. C, b) during a prolonged diastolic pause. The undulating trace during a prolonged diastolic pause of course indicates corresponding changes in the size of the ventricle, quite distinct from rhythmic contractions. When the undulation is at its highest, it shows that the ventricle is more contracted, and when the wave dips again, it shows that the ventricle is somewhat more relaxed. This change in the ventricle I hope to show is a true muscular contraction, a prolonged but varying spasm. This persistent but varying spasm, induced by ammonia, explains Fig. 2, C and D, where there occurs a series of rhythmic contractions, gradually increasing in amplitude and then diminishing again.

When the persistent spasm is most marked, the diastolic dilatation following each rhythmic contraction grows less, and the trace becomes small. When the persistent spasm grows less, then the diastolic dilatation following each rhythmic contraction increases, and the amplitude of the trace becomes greater, and so the amplitude of the trace grows from time to time larger, and then again grows less. When the slow persistent contraction induced by the alkali is least marked, the diastolic dilatation after each rhythmic contraction becomes greater, and the subsequent rise in the next rhythmic trace is increased, but when the persistent contraction from the alkali increases again, then the diastolic dilatation after each rhythmic contraction is lessened, and the rhythmic trace is much diminished. Indeed, the trace given in the above diagram corresponds in character to the trace recorded in Fig. 1. Indeed, C and D, in Fig. 2, correspond to Ba, Cb, Da, in Fig. 1, only in Fig. 2, whilst changes occur corresponding to those in Fig. 1, the rhythmic contractions continue.

After all rhythmic contractions have ceased, the trace gradually falls and attains the position in regard to the base-line it held before the ammonia was administered.

To what is this slow, persistent, varying contraction, occurring in long, diastolic pauses, due?

Is it due to a mere physical change which causes a shrinking in the ventricle, or is the contraction vital, and, indeed, a form of muscular contraction?

I hope to show that the slow diastolic contraction is vital—is a true muscular contraction:—

1st. These substances, hydrate of potash, hydrate of soda, and hydrate of ammonia, produce no such effect on the dead ventricle.

2nd. If the contraction were a mere physical change, the trace should remain at the same level during the whole diastole.

The great fall of the trace after a rhythmic contraction might possibly be explained on the supposition that, on the sudden relaxation of the ventricle, the rush of blood into the ventricle dilates it considerably, owing to some increase in its elasticity induced by the alkalies, to be followed by slow retraction of the ventricle.

This explanation, however, is not feasible, since (see Fig. 1, E aa) after a rhythmic contraction a marked fall occurs, when so slight has been the rhythmic contraction that its relaxation would not admit of sufficient blood to distend the ventricle. When the rhythmic contraction relaxes it causes, likewise, relaxation of the persistent spasm induced by the alkali.

Further, in a long diastolic pause the trace undulates (see Fig. 1, C b, D, Fig. 4, B), and on examining the ventricle, one sees that it slowly dilates, first at the apex or base, and then the dilatation extends to the rest of its walls; finally the whole of it again slowly contracts, to again relax, so that the condition of the ventricle during diastole is seen to be a true muscular contraction, and not a mere physical phenomenon.

The alkali evidently induces a persistent contraction or spasm in the muscular structure of the ventricle, but this contraction slowly remits, and so gives rise to an undulating trace under a small dose. After a rhythmic contraction this spasm completely relaxes, so that the trace falls to its original position to the base-line; after a larger dose the spasm relaxes to a less and less degree, so that after each rhythmic contraction the fall grows less and less. (See Fig. 1, E b.)

If it should prove that a tonus exists in the muscular substance of the heart, I suggest that the spasm induced by these alkalies is an increase of this tonicity; but as this spasm can be induced in the apex of the ventricle, a part free from nerves, so it follows that the tonicity must be independent of nervous agency.

Dr. Chas. Richet (*Lancet*, May 21, 1881) argues that contracture is only an exaggerated tonicity of the muscles, and if so, this condition induced in the ventricle may be compared to contracture.

Hydrate of Soda.

Hydrate of soda affects the ventricle just like ammonia, as Trace 3 shows.

Hydrate of Potash.

Hydrate of soda and hydrate of ammonia induce a spasmodic contraction in the muscular tissue of the heart, and after still larger doses

Fig. 6 A.

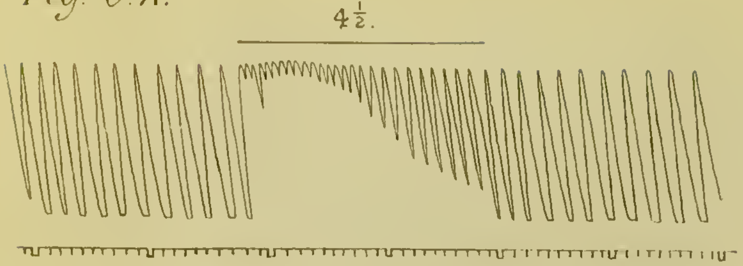


Fig. 6, C.

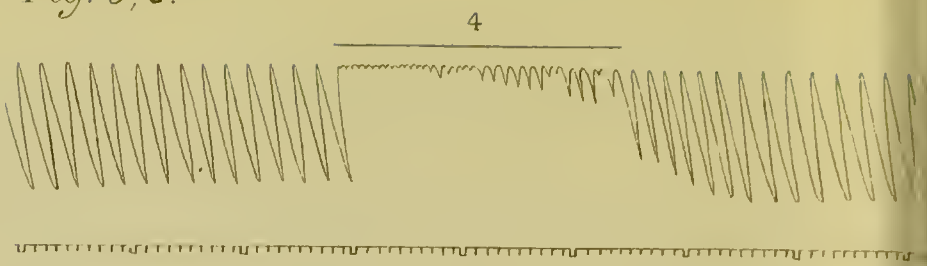


Fig. 6 B.

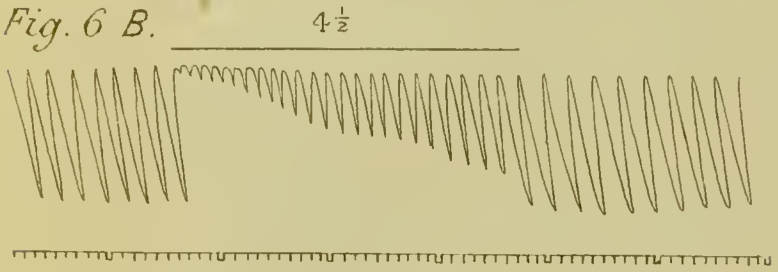


Fig. 6, D.

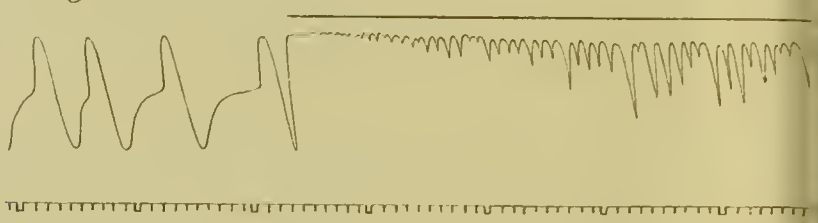


Fig. 6, F.

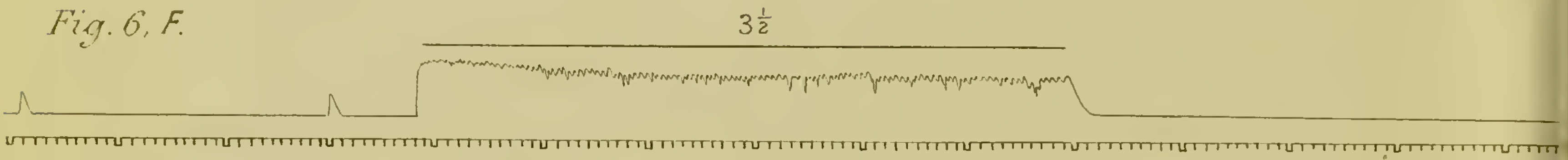


Fig. 6, α.

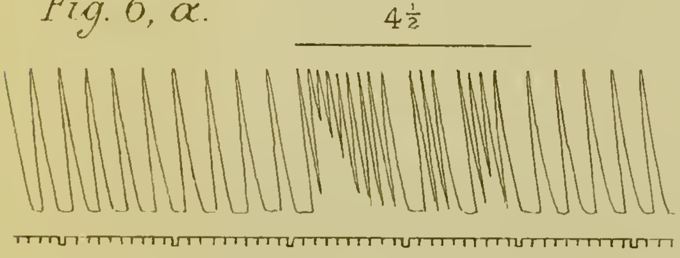


Fig. 6, β.

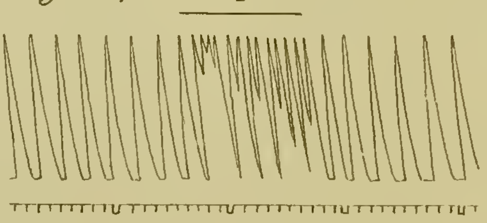


Fig. 6, γ.

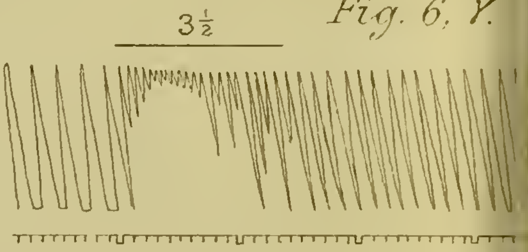


Fig. 7, A.

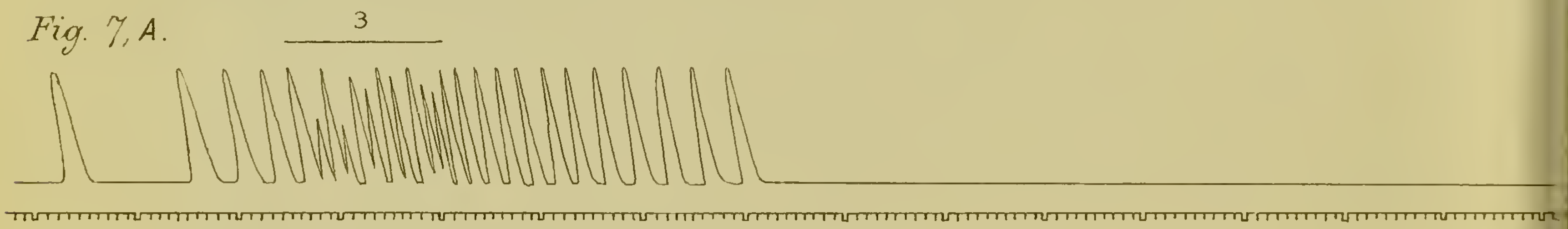
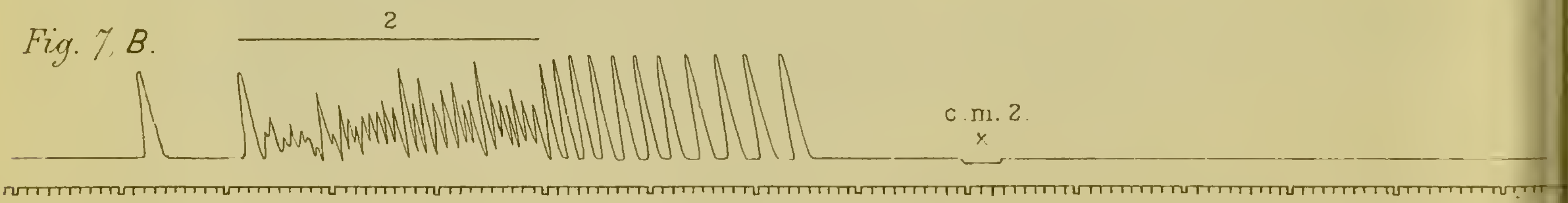


Fig. 7, B.



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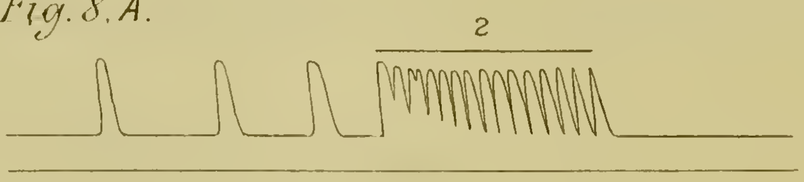


Fig. 8, B.

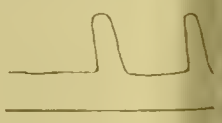
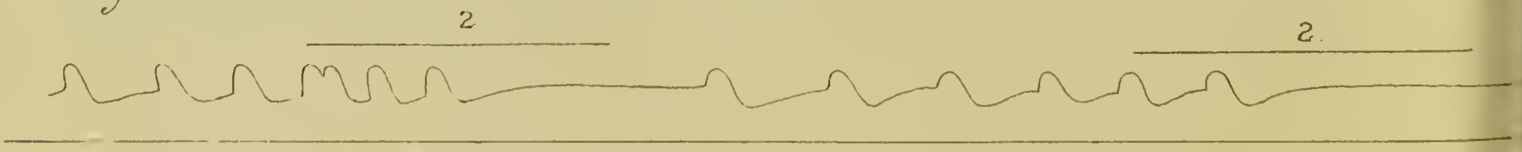
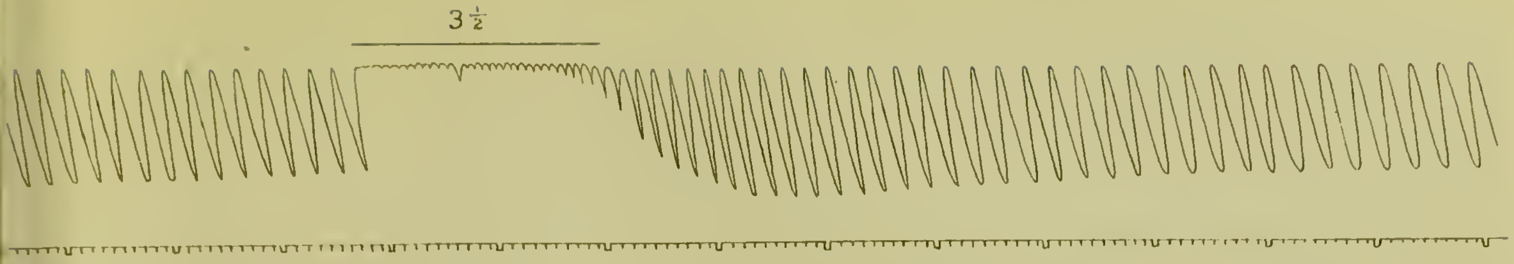
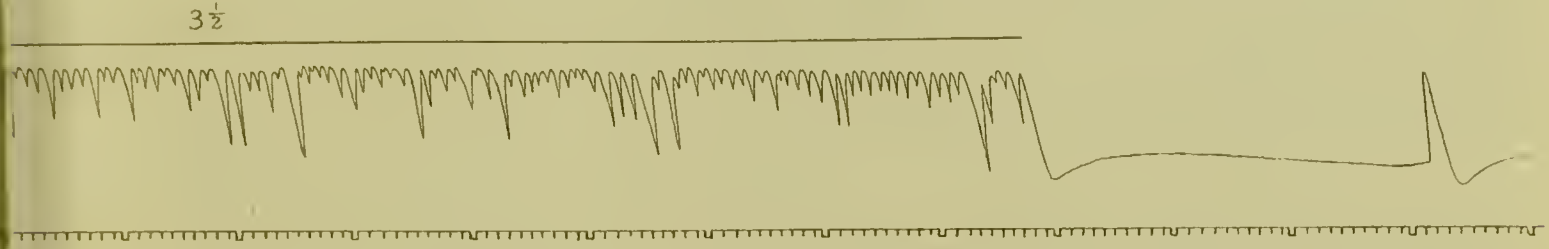


Fig. 8 C.







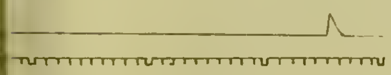


Fig. 6, E.

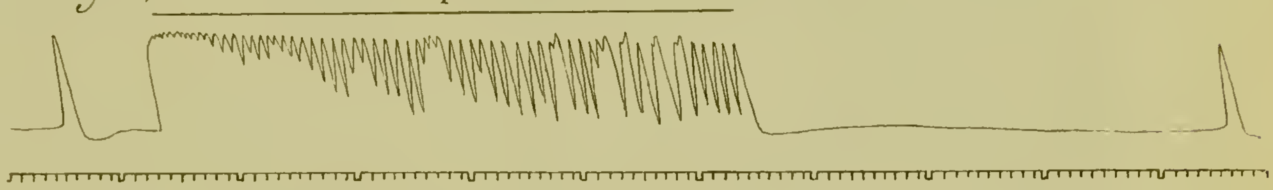
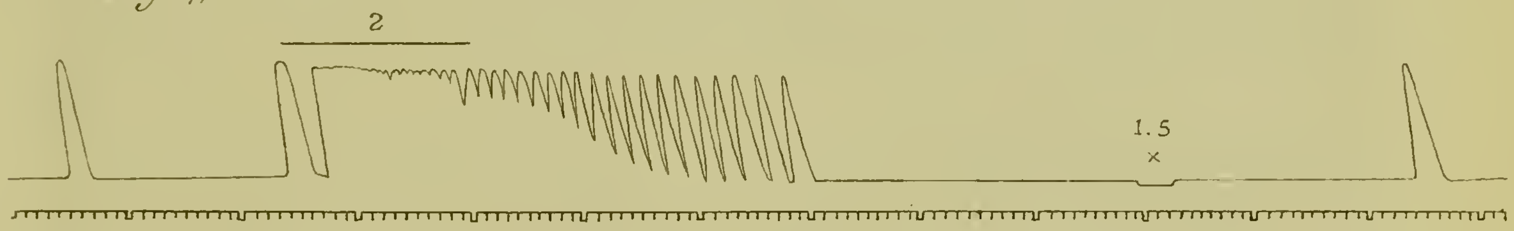


Fig. 7, C.



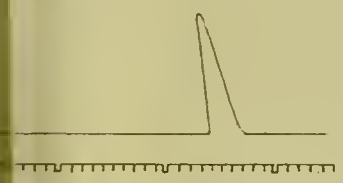
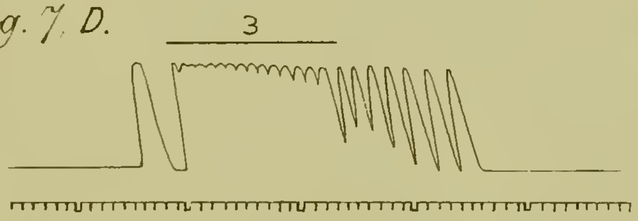


Fig. 7, D.



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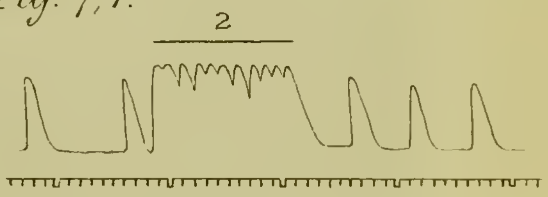


Fig. 7, E.

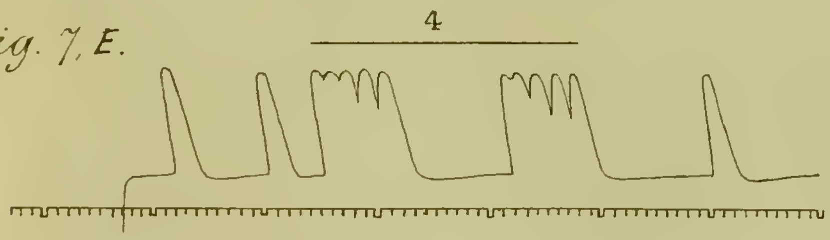
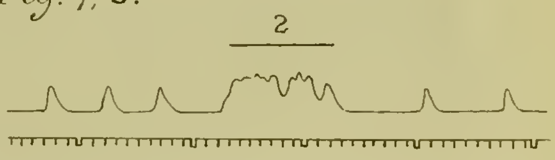
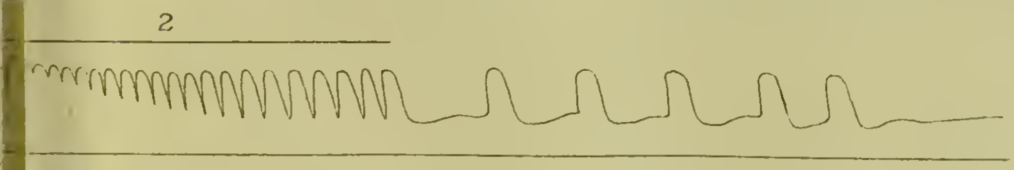
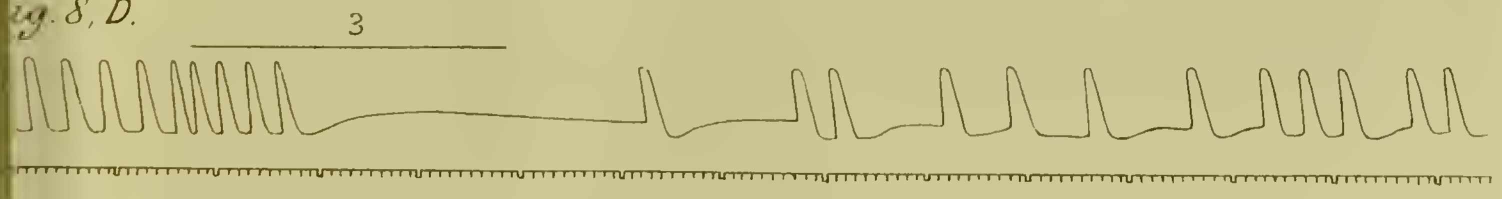


Fig. 7, G.





ig. 8, D.



F Huth, Lithr Edinr

all muscular contractility, rhythmic and spasmodic, ceases, and the heart dilates again, the trace falling towards the base-line till it reaches the same position it held before the addition of the alkali. In other words, soda and ammonia at first induce spasm and later weaken and paralyze muscular contractility.

Potash, also, induces spasm followed by paralysis. The paralytic action, however, becomes much sooner apparent. Shortly after the trace becomes modified the heart fails to contract completely with each rhythmic contraction, and, consequently, the trace does not rise so high as before the addition of the potash.

Potash is, indeed, a far more powerful paralyser than either soda or ammonia. The effects of hydrate of potash are well seen in Figs. 4 and 5.

A small quantity of potash, like soda and ammonia, prolongs each contraction and makes its summit rounder (see Fig. 5, B). As soon, however, as the potash induces the spasmodic condition, the rhythmic contractions lessen in height and become pointed once more (see Fig. 5, C). Indeed, the ventricle can be seen to empty itself much less completely. In some instances the beats become very irregular in force.

When the ventricle in Roy's tonometer is stimulated by the continuous application of faradization, it gives a trace very similar to a tetanus curve of the skeletal muscles when the excitations are not very numerous. The ventricular trace, however, differs from a true tetanus trace in this respect. In a tetanus trace the contractions are as numerous as the excitations, or in other words, as the breaks in the current, but it is not so with the ventricle. I used a Du Bois Reymond's induction coil, and the interruptions were very frequent, far more frequent than the ventricular contractions[1].

Soda and ammonia increase the readiness with which this condition can be induced, as is seen in Figs. 6 and 7, Pl. XIII., where I give, first the effect of faradization alone, and then of faradization after the heart has been drugged with a solution of hydrate of soda.

These tracings show that soda and ammonia greatly enhance the effects of faradization. In other words, soda and ammonia increase the irritability of the ventricle.

This increased irritability persists even when the ventricle has grown very weak, almost to the point of stopping, for even then, as seen in

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[1] In the charts, the line above the trace indicates the time the faradization was continued, and the figure above the line the point at which the induction coil stood.

Fig. 6 F, Fig. 7 F, the ventricle when faradized gives a tetanus-like trace, though the point to which it rises above the base-line is only a little higher than that reached in a spontaneous rhythmic contraction, and far less high than before the addition of either alkali. The heart, though weak, is made irritable by the alkali. The alkali induces a condition similar to that occurring in disease in the nervous and muscular tissues, and termed, by the old writers, irritable weakness.

Prolonged faradization of course weakens the ventricle, and so the persistent spasm becomes weakened as well as the rhythmic contractions; hence, after prolonged faradization the trace sinks nearer the base-line, and as the heart recovers from the weakening effect of faradization, the trace again gradually rises higher and higher above the base-line. This effect of faradization on the trace favours the suggestion that the diastolic rise after ammonia or soda is due to muscular contraction, and not to a mere non-vital physical change.

Prolonged faradization affects a heart poisoned by potash differently from a heart poisoned by either soda or ammonia.

With some hearts there occur after potash for a short time a similar effect to that induced by the other alkalies; that is to say, the ventricle is more irritable, and the trace remains raised longer than before the addition of potash. Soon, however, when the effects of potash become marked, and when diastolic contraction is induced, potash has the opposite effect, and the ventricular irritability is diminished, and continuous faradization weakens the ventricle, and the trace sooner falls towards the base-line than before the potash was added. A little later the faradization arrests all contractions whilst it is continued, but these return when the faradization is discontinued. (See Fig. 8, C D.)

In other cases the heart at once, after potash, grows less influenced by faradization, and in a short time the contractions are arrested by faradization.

EXPLANATION OF PLATES.

Plate XII.

Fig. 1.—*Ammonia.*—A. Before the addition of ammonia to the blood.
B. After the addition to the blood of successive doses of 0·1 c. c. of the 1 p. c. ammonia solution repeated seven times.
C. After 1·4 c. c. of the solution.
D. „ 2·6 „
E. „ 3·8 „

Fig. 2.—*Ammonia.*—A. Before the addition of the ammonia solution.
B. After the addition of 0·8 c. c. of the ammonia solution.
C. „ 1·8 „
D. „ 3·0 „
E. „ 2·2 c. c. of the ammonia solution 32 minutes after the first dose of ammonia.

Fig. 3.—*Soda.*—A. Before the addition of 1 p. c. soda solution.
B. After the addition of 1·2 c. c. of the soda solution.
C. „ 2·0 „
D. „ 4·0 „
E. „ 4·6 c. c. (in all) „
F. „ 7·0 c. c. of the soda solution.

All contractions then ceased and the heart gradually dilated, the curve falling until it ultimately touched the base line.

Fig. 4.—*Potash.*—A. Before the addition of potash.
B. After the addition of 1·0 c. c. of the 1 p. c. potash solution.
C. The effect of additional doses of the potash solution.

Fig. 5.—*Potash.*—A. Before the addition of the potash solution.
B. After the addition of 2·5 c. c. of the potash solution.
C, D. Effects of additional doses of the potash solution.

PLATE XIII.

Fig. 6.—*Soda.*—α, β, γ. Before the addition of the soda-solution, effect of faradization of ventricle.

The lines above the curve indicate the duration of the faradization, the numbers above the line the distance between the secondary and primary coils, *i.e.*, the relative intensity of the current.

A. After the addition of 2·0 c. c. of the soda solution.
B. „ 3·0 c. c. of the soda solution; 14 minutes after the first dose of soda.

No more of the soda solution was added to the blood, but the following tracings were taken at intervals.

C. Eight minutes after the first dose of soda.
D. About 27 minutes after the first dose of soda.
E. „ 36 „ „
F. „ 63 „

Fig. 7.—*Ammonia.*—A, B. After the addition of small doses of the ammonia solution.

C. After the addition of 1·3 c. c. of the ammonia solution in successive doses ; tracing taken about 11 minutes after the first dose of ammonia.

D, E. Additional doses of ammonia.

F. After 3·2 c. c. of ammonia solution ; 36 minutes after the first dose.

G. At a still later period.

Fig. 8.—*Potash.*—A. Before the addition of the 1 p. c. potash solution.

B. After the addition of the 1 p. c. potash solution in successive doses, 3·0 c. c. in all.

C. After the addition of 3·8 c. c. in all.

D. Tracing taken from another heart after the addition of 50 *minims* of the potash solution.

Journal Physiology.

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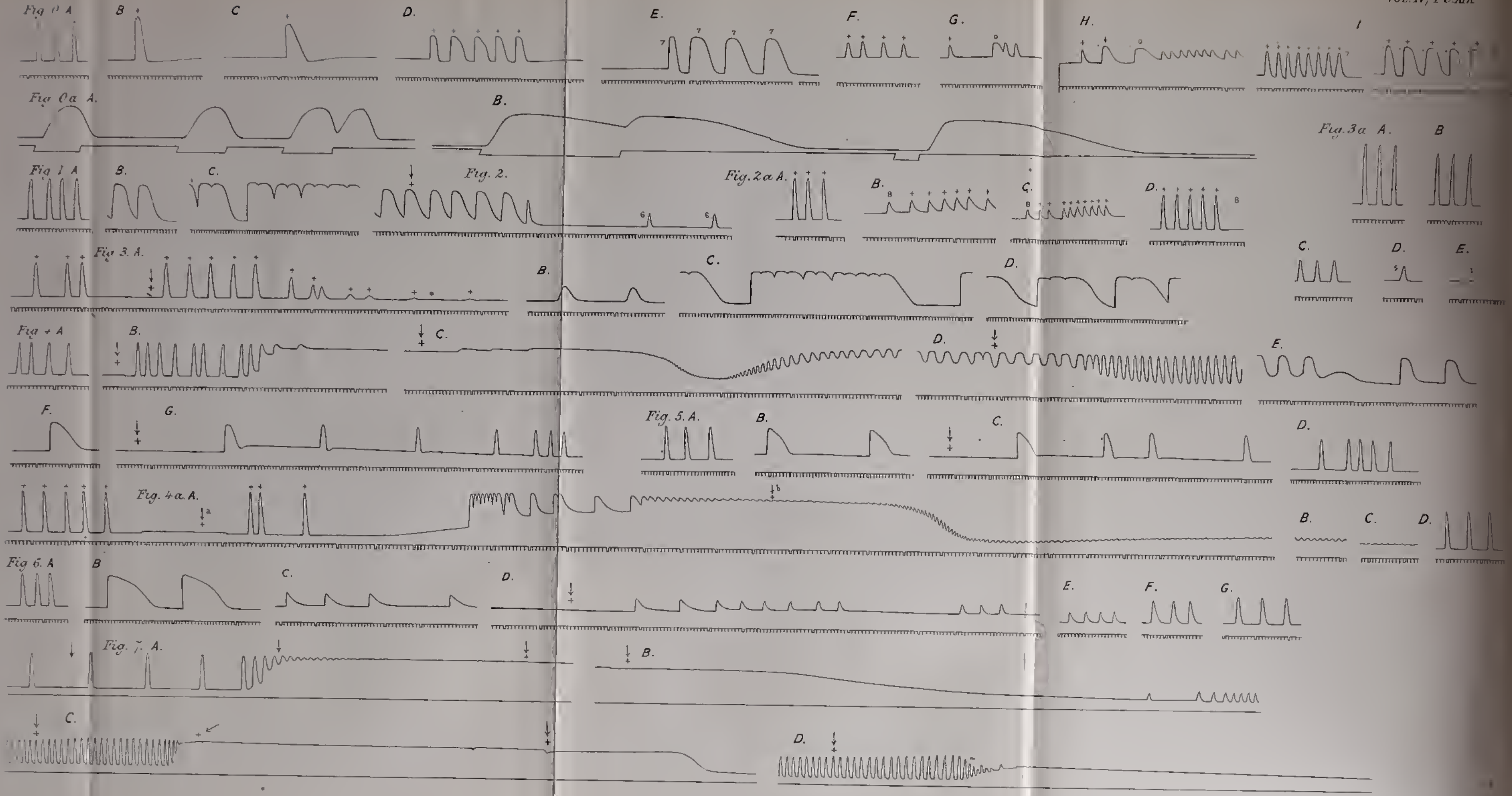

CONCERNING THE INFLUENCE EXERTED BY EACH OF THE CONSTITUENTS OF THE BLOOD ON THE CONTRACTION OF THE VENTRICLE. By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.* Plate XIX.

In this paper I record experiments designed to ascertain the influence each constituent of the blood exercises on the contraction of the ventricle.

By "saline solution" I mean the ordinary 0·75 per cent. solution of sodium chloride. "Blood mixture" was made from dried bullock's blood dissolved in water to represent normal blood, and this I diluted with five parts of saline solution.

In each experiment I always used 100 c. c. of saline solution or of blood mixture. The tracings were taken with Roy's tonometer, and run from left to right. The experiments were made in April and May. The ventricle was tied on the cannula as nearly as possible in the auriculo-ventricular groove. A figure or * over the trace indicates that the contraction was excited by faradaic stimulation, the figure showing the position of the secondary coil.

When the circulating fluid consists only of "saline solution" the ventricular beats undergo the following changes (see Fig. O, Oa, 1, 5, 6). The contraction at first becomes more complete if the ventricle empties itself imperfectly. The trace soon becomes broader, its summit rounder (see Fig. O, B), at the same time there is slight diastolic contraction (persistent spasm). Next, the period of relaxation becomes greatly prolonged (see C; Fig. 5, B, and Fig. 6, B), and the whole trace is permanently raised higher above the base line.

During these changes affecting the expansion of the ventricle the contraction is very little altered, being rather accelerated, see Fig. Oa (trace taken with quicker rate), where A shows the trace with blood mixture, B the trace after saline solution was substituted for blood mixture. The dip in the abscissa shows the time the make, and the rise the time the break induction shock was delivered. The secondary coil stood at 3. These effects, therefore, are very similar to those produced by veratria.

Next, this prolongation of the ventricular dilatation grows less, at the same time the trace rises still higher above the base line (see Fig. O, D). In a series of contractions excited after a standstill of some 50 or 60 seconds the first contraction dilates more rapidly than the subsequent ones. This is still better seen in E, taken from another experiment.

These changes in the trace increase, that is, the whole trace rises higher and higher above the base line, the amplitude and duration of each contraction grows less, and the dilatation often becomes less prolonged (see F). At the same time the ventricle undergoes another curious change. After the contractions have become weakened the duration of the contraction and the slowness of dilatation are influenced by the strength and duration of the stimulus. For instance, in some cases if, in the case of a ventricle not contracting spontaneously, the coil be moved from, say, 8 to 0, the contraction is prolonged and followed by spontaneous contractions (see G), where the contraction with * over it was excited with the coil at 8, the contraction with ⁰ over it with the coil at zero.

Again, if the faradaic excitations be continued till the trace has nearly completed its rise, the contraction is much prolonged. See H, where the first contraction was excited by a momentary excitation, the connection in the secondary current being made and broken as speedily as possible, and the coil standing at 8. In the second contraction the faradaization was continued to the point opposite the dot, the coil still standing at 8. In the third contraction the coil stood at 0, and the faradaization was continued to the point opposite the dot. The second contraction is much more prolonged than the first, and the third than the second; and, moreover, the third contraction is followed by a series of spontaneous beats. This is still better seen in I, taken from another experiment. In the first series each contraction was excited by the shortest duration of faradaization possible, the secondary current being made and broken as speedily as possible. In the second series of contractions the faradaization was continued to a level with the dot. In each series the coil stood at 7. This effect of an increase in the strength, or duration, and number of stimuli does not occur in a heart fed with blood, nor, in the early stages, with saline. With a stronger solution of sodium chloride—as, for instance, 1·5 per cent.—all the above changes occur earlier.

When the dilatation is greatly prolonged, in many cases the dilatation is completed at the base, whilst the rest of the ventricle remains quite contracted, and then the dilatation extends gradually to the apex. Sometimes, before the dilatation has spread far down the ventricle, the dilated portion contracts again, and there ensues a partial fusion of the excursions in the trace ; in fact, we get an imperfect tetanus, the fusion being due, not to increased frequency of the contractions, but to their great prolongation, a second contraction beginning before its predecessor is finished (see Fig. 1, C).

Blood, even in small quantities, obviates the changes with saline solution. Thus fresh or dried blood diluted with three or four parts of saline does not induce the character of contraction occurring with saline, and yet the blood mixture contains as much soda salts as saline solution.

On one occasion I dissolved the dried blood in "saline solution," so that the blood mixture contained an excess of soda salts, and this mixture did not prolong the ventricular dilatation. In other experiments I added to the blood mixture one, two, and three times its own quantity of saline, and with the larger additions only very slightly increased the duration of the contractions. On another occasion I added to the 100 c. c. of saline solution 25 c. c. of blood mixture, and almost entirely removed the effect of the saline, the contractions becoming of much shorter duration, and the dilatation much more rapid.

Blood mixture not only obviates the changes occurring early with sodium chloride, but also the later changes. This effect is seen in Fig. 2a. In about 60 minutes, with saline, the contractions had grown very weak, the dilatation remaining prolonged (see B). On substituting blood for saline, at first the contractions became still weaker, but the dilatation became more rapid. The contractions then slowly improved, and soon the dilatation became as quick as at first, and in about 40 minutes after the saline was changed for blood mixture the contractions had become almost as good as at the beginning of the experiment. On the addition of blood solution, or on replacing saline with blood solution, the contractions at first become much less frequent and weaker (see Fig. 2), but in a short time the contractions became good again.

If sodium chloride is added from time to time to the blood mixture till enough is employed to arrest the ventricle, still we do not produce the character of trace that occurs when the ventricle receives only saline solution. This is well shown in Fig. 3. At A is shown the effects of 5 c. c. of 20 per cent. solution of sodium chloride. This quickly weakened and arrested the ventricle. I then restored contractions by adding 50 c. c. of water to the circulating blood mixture and again weakened the ventricle by adding 2 c. c. of 20 per cent. chloride of sodium solution (see B). I then substituted for the blood mixture saline solution, and got the characteristic saline solution trace, the dilatation becoming greatly prolonged (see C and D). These last tracings will show the effect of a prolongation of the contraction on the production of tetanus, for in C and D there is considerable fusion of the contractions, not by increasing their frequency, but by prolonging the duration of each beat, so that one begins soon after its predecessor is nearly completed.

In Fig. 3a I give the effect of repeated smaller additions of sodium chloride solution to the blood mixture, and it will be seen that, administered in this way, we do not produce the change that occurs with simple saline solution. Blood, then, obviates the changes occurring in the contractions of a ventricle receiving only saline solution, and the question arises, Which constituent of the blood has this property?

I find that a small quantity of white of egg completely obviates the changes occurring with saline solution. I first took tracings with blood mixture; I then replaced the blood with 100 c. c. of saline, and got the usual great prolongation of dilatation. I then added to the saline 25 c. c. of white-of-egg mixture, composed of one part white of egg in two parts of water. In two minutes the contractions became exactly as they were when the heart was supplied with blood mixture. White of egg consists of albumins and chloride of potassium and sodium, chiefly chloride of potassium. It is obvious in the preceding experiment that the effect of white of egg could not be due to the sodium chloride. The power to obviate the great prolongation of the dilatation occurring when the ventricle was supplied with saline solution must then be due to the albumins or the potassium chloride.

I find that potassium chloride in small quantities, much smaller than exists in serum, will completely and speedily obviate the character of the trace occurring with saline solution, and give a trace in all respects like that occurring when the ventricle is supplied with blood mixture. This is well shown in Fig. 5. I first took a tracing with blood mixture (see A), and then replaced the blood with 100 c. c. of saline solution. After about 15 minutes I obtained the usual great prolongation of the ventricular dilatation (see B).

I then added to the 100 c. c. of circulating saline 0·7 c. c. of 1 per cent. solution of potassium chloride (see C) at the point indicated by the arrow; very speedily the contraction became modified, the dilatation becoming much shorter. Ten minutes after the addition of the potassium chloride the contraction became just like those at the beginning of the experiment when the ventricle was supplied with blood (see D). From numerous experiments I find that from 0·6 c. c. to 1 c. c. of 1 per cent. solution of potassium chloride to the 100 c. c. of saline solution is sufficient to remove the prolongation of dilatation occurring with saline solution.

Like blood mixture small doses of chloride of potassium obviate the changes occurring in the later stages of a ventricle fed with saline solution. This is well seen in Fig. 6. After the trace had become greatly weakened (see C), the dilatation remaining greatly prolonged, I added 1 c. c. of 1 per cent. solution of potassium chloride. This speedily altered the trace; it accelerated the dilatation and brought the whole trace nearer the base line. The contractions then grew slowly stronger, and in 34 minutes they were nearly as good (see G) as at the beginning of the experiment, when the ventricle was supplied with blood (see A). On another occasion I restored good contractions by the addition of 0·7 c. c. of 1 per cent. solution of potassium chloride.

Phosphate of potash in smaller doses than exist in the blood likewise obviates the prolonged relaxation occurring with saline solution. I employed a 1 per cent. solution of potassium phosphate from which the greater part of water of crystallization was driven off by placing the salt over a water bath for two hours at a temperature of 212° Fahr. I found that 0·6 c. c. of this solution greatly and speedily shortened the duration of the dilatation occurring with saline solution, and 1·5 c. c. restored the trace to its condition when the ventricle was supplied with blood.

Phosphate of soda, however, does not remove the prolonged dilatation with saline solution, even with quantities largely in excess of the amount of sodium phosphate in the blood. The effect, then, in potassium phosphate is due to the potassium and not to the phosphoric acid.

Probably any potassium salt is sufficient to obviate the prolonged dilatation occurring when the ventricle is fed with saline solution only, for I have produced normal contractions by adding to the saline solution potassium chloride, potassium phosphate, potassium sulphate, potassium citrate, potassium bicarbonate, potassium chlorate, potassium nitrate, potassium acetate, and potassium carbonate.

With all of these 1 to 2 c. c. of one per cent. solution added to 100 c. c. of saline solution was sufficient to give a normal contraction.

As an illustration of the effect of potassium salts, I describe the following experiment : I first took a trace with blood mixture. I then replaced the blood with saline solution and obtained the saline trace. I then added 1·5 c. c. of potassium chlorate to the saline, and soon the trace became normal, like that with blood mixture. I then again supplied the ventricle with saline solution, and a second time obtained the saline trace, and then I added 1·5 c. c. of 1 per cent. solution of potassium nitrate and produced a normal contraction. I then a third time supplied the heart with saline solution, and a third time produced the saline trace. I then added 1·5 c. c. of 1 per cent. solution of potassium acetate and produced normal beats. I then a fourth time supplied the ventricle with saline, and a fourth time obtained the saline trace. I then added to the saline solution 1 c. c. of 1 per cent. solution of potassium carbonate, and produced normal beats in all respects like those at the commencement of the experiment when the ventricle was supplied with blood mixture.

I next tested the effect of serum albumin, free from potassium salts, on the saline trace. I dialyzed serum of lamb's blood for four days, and dissolved the precipitated albumin by adding to 100 c. c. of the dialyzed serum an equal quantity of $1\frac{3}{4}$ per cent. of sodium chloride solution, and added 1 c. c. of 1 per cent. solution of bicarbonate of soda. I first took a trace with blood mixture, and then replaced the blood with 100 c. c. of saline solution. The usual great prolongation of dilatation ensued, and I then added to the saline solution 25 c. c. of the serum mixture, but without modifying the trace in the smallest degree. I then added a second 25 c. c. of serum mixture, but still the great prolongation of the ventricular dilatation continued. I then added 1 c. c. of 1 per cent. solution of potassium chloride, and, as usual at first, the contractions grew weaker, but they soon regained their strength and became in all respects like the contraction with blood, the dilatation becoming natural. It is evident, therefore, that the albumin in serum will not obviate the prolonged dilatation that occurs when the ventricle is fed with saline solution alone.

It appears, then, that a small quantity of potassium salt is necessary for the prompt dilatation of the ventricle. It is singular that whilst necessary for the speedy dilatation of the ventricle, potash salts appear less necessary for the proper contraction of the ventricle ; for with saline solution the contraction occurs with much the same rapidity as with blood, and the dilatation only is greatly retarded.

I next tested the action of distilled water on the ventricle. I first took a trace with blood mixture, and then replaced this with 100 c. c. of distilled water. Water throws the ventricle into complete and permanent systole; in fact, it affects the cardiac like the skeletal muscles, and induces water rigor. This effect of water is removed by adding sodium chloride to the water. The effects of water, and the counter effect of sodium chloride, are well shown in Fig. 4. A shows the trace with blood mixture. At B is seen the rapid effect of water, the ventricle being thrown into complete and permanent systole. At C is shown the antagonizing effect of sodium chloride, which dilated the ventricle and restored to it spontaneous contractility. After a time the trace presented the usual character with a ventricle fed with saline—that is to say, the dilatation became greatly prolonged. I then added a small quantity of white of egg mixed with water, and in a few seconds the ventricular contractions became just as they were with blood mixture.

Fig. 4a also shows the effect of water and the antagonizing action of sodium chloride.

White of egg dissolved in water affects the ventricle just like water. I diluted white of hen's egg with four times its quantity of water and filtered it. I then took a trace with blood mixture and replaced this with 100 c. c. of the white-of-egg mixture, and produced almost at once complete arrest of the ventricle in complete systole. I then added to the albumin mixture 2 c. c. of 10 per cent. solution of sodium chloride, and in about 70 seconds the heart dilated, and the trace fell to its old position to the base line. Soon after this slight contractions began and increased in strength, the trace again rising higher above the base line—in other words, some tonic spasm set in; soon the trace again fell towards the base line, the tonic spasm ceasing, and then occurred contractions similar to those induced by adding sodium chloride to blood mixture. On replacing the albumin mixture with blood mixture good contractions returned, similar to those at the beginning of the experiment, but the contractions were rather less complete.

We see then that water induces in the ventricle tonic spasm (water rigor), that an addition of white-of-egg albumin will not prevent this, but that the spasm is obviated by sodium chloride.

If some time elapses after the production of water rigor before the addition of sodium chloride solution, then there occurs no return of the beats nor fall in the trace, indeed no effect on the water rigor. I produced water rigor, and about seventeen minutes after I added 3 c. c. of 20 per cent. sodium chloride solution, and in six minutes another 2 c. c. and continued the observation twenty-two minutes after the first addition of sodium chloride solution, but the water rigor remained unchanged. On another occasion I allowed six minutes to elapse after the induction of water rigor before I added 3 c. c. of 20 per cent. solution of sodium chloride. The water rigor remained unaffected, though I continued the observation eleven minutes after the addition of sodium chloride. On another occasion I added 3 c. c. of 20 per cent. solution of sodium chloride to the water 150 seconds (2·5 minutes) after the heart was first supplied with distilled water. This restored feeble contractions, and as usual the trace fell to its natural position to the base line (see Fig. 4a). But the contractions remained very feeble, and then grew weaker and had almost ceased in thirteen minutes after the addition of sodium chloride. I then replaced the water and sodium chloride solution with blood mixture, and in 90 seconds spontaneous contractions began and became very good in nine minutes.

It is evident, therefore, that blood is more efficient in restoring the contractions after water rigor than simple saline solution. But if too long an interval elapses between the production of water rigor and the substitution of the blood mixture for water, the water rigor remains unaffected by the blood. I induced water rigor, eleven minutes after the use of water I replaced it by blood solution, but the water rigor remained unaffected.

We see, therefore, that water, or water and white of egg, first induce spasm—a condition closely similar to rigor mortis—and, after a little while longer, true rigor mortis, the ventricle becoming white and opaque, and then neither sodium chloride nor blood mixture can cause the ventricle to dilate and to resume contractility.

Is the action of simple water on the heart merely physical, and does chloride of sodium simply prevent these physical effects?

To see how far sodium chloride acts merely physically, I used syrup 1·3 in two parts. First I produced water rigor, and then added to the circulating water 1 c. c. and subsequently another c. c. of the syrup without in any degree lessening the water rigor. On another occasion I substituted for the blood mixture 100 c. c. of distilled water, to which I had previously added 3 c. c. of this syrup, but the water rigor occurred in spite of this and was removed by the addition of 3 c. c. of 20 per cent. sodium chloride solution.

Phosphate of soda will, however, remove water rigor. On two occasions I removed it—the heart fully dilating—with 3 c. c. of 20 per cent. solution added to 100 c. c. of circulating water. In one experiment no contractility returned, either spontaneously or with strong faradaic excitation; on the other occasion, slight and frequent contractions returned, but ceased in about seven minutes, and were not restored by 1 c. c. of potassium chloride 1 per cent. solution, but were by substituting blood mixture.

We see that water produces water rigor, *i.e.* tonic contraction of the ventricle, and that sodium chloride prevents this effect. But with saline solution while contraction is normal, dilatation is greatly prolonged. Is this slow dilatation of the ventricle due to the effect of water being only partially antagonized by saline solution? If this suggestion is correct the stronger solution of sodium chloride should quicken the dilatation and make it normal. But this is not the case, for I have used solutions containing 1, 1·5, 1·7, and 2 per cent. and yet never obtained a normal trace but the saline trace, the effects occurring much sooner than with 0·7 solution. A stronger solution than 2 per cent. quickly arrests the ventricle in diastole.

I find that potassium chloride in physiological doses does not remove water rigor. I made three experiments, and after the production of water rigor added to the circulating water 1 c. c. of 1 per cent. of potassium chloride without any effect. This indeed one would expect, for white of egg, which contains a fair quantity of potassium chloride, does not prevent water rigor.

I next experimented to learn how far chloride of potassium is able in larger doses to prevent the effects of water (water rigor). When administered with blood, potassium salts are far more poisonous than soda salts. 1·5 c. c. of 10 per cent. solution of potassium chloride added to 100 c. c. of blood mixture quickly arrests the heart in diastole. Again, we have seen the striking effect of a quantity less than that present in the blood on the prolonged dilatation of the ventricle supplied with sodium chloride solution.

Although potassium chloride is powerful in arresting the ventricle in diastole, yet it cannot prevent water rigor. I first took a trace with blood mixture, and then substituted 100 c. c. of distilled water for the blood and quickly produced water rigor. Then, before spontaneous contractions were abolished, I added 3 c. c. of 1 per cent. potassium chloride solution, and in about a minute and a half another 2 c. c., but the ventricle remained firmly contracted and did not beat spontaneously, nor could contractions be induced by a strong induction shock.

On another occasion, after taking a tracing with blood mixture, I replaced it with 100 c. c. of distilled water, to which I had added 5 c. c. of 10 per cent. solution of potassium chloride; but this neither prevented nor retarded the onset of water rigor, and immediately this occurred I added another c. c., and in about a minute 2 c. c. more, but without removing, or even lessening, the contraction of the ventricle. I then replaced the potassium chloride solution with blood and the ventricle soon dilated, and the trace fell to the base line, and shortly after this good spontaneous beats returned. I replaced the blood with 100 c. c. of water, to which I had added 5 c. c. of 10 per cent. solution of potassium chloride; but again water rigor speedily set in as complete as with water alone. Immediately the ventricle became quite contracted. I added another 5 c. c. of 10 per cent. solution, but did not at all lessen the contraction. I then replaced the potassium chloride solution by 100 c. c. of 0·75 per cent. solution of sodium chloride solution, and the ventricle quickly dilated, the trace falling to the base line, and soon good contractions ensued. I then added to the saline solution 2 c. c. of 10 per cent. solution of potassium chloride, and in a few seconds the ventricle became arrested with some persistent spasm, which, however, soon disappeared (see Fig. 7).

On another occasion, after taking a trace with blood mixture, I substituted for the blood 100 c. c. of 0·75 per cent. solution of potassium chloride, and threw the ventricle into strong systole, which persisted six minutes, when I replaced the chloride of potassium solution by blood. In about 3 minutes contractions returned, and when these had become fair I again replaced the blood by potassium chloride 0·75 per cent. solution, and this time the contractions grew rapidly weaker and the ventricle stopped beating in diastole.

At another time after a blood trace I replaced the blood by 0·75 per cent. solution of potassium chloride. I produced partial systole, and the trace fell towards the base line, but remained higher than with blood. I replaced the potassium chloride solution by blood and got back contractions, and again replaced the blood by 0·75 per cent. solution of potassium chloride. The contractions grew less and less complete and the ventricle stopped in diastole; but immediately after the ventricle slowly contracted again and passed into a state of persistent spasm. I again restored spontaneous beats by means of blood mixture, and again replaced the blood by 100 c. c. of 0·75 per cent. solution of potassium chloride and again obtained the same result, the beats growing weaker and the ventricle stopping in diastole, but then slowly passing into persistent spasm.

It appears, then, that neither physiological nor toxic doses of potassium chloride will obviate the contraction induced by water.

Part of the persistent contraction of these last experiments is probably due to the potassium chloride, for though with the addition of small doses at a time, the ventricle becomes arrested in diastole without the production of any persistent spasm, yet if a large dose be added at once the contractions grow weak or are arrested, and then occurs a considerable amount of persistent contraction, which, however, speedily declines.

We see, then, that water alone induces persistent spasm, and that this is not prevented by potassium chloride nor by albumin (white of egg), but is obviated by sodium chloride. Sodium chloride solution alone, however, is accompanied by considerable prolongation of the dilatation of the ventricle, so that, with the heart beating at its normal frequency, there would occur a considerable amount of fusion of the beats, and the heart's work would be considerably interfered with. Minute doses of potassium salts, smaller even than exist in the blood, when added to the saline solution greatly accelerate the dilatation of the ventricle and give a normal beat in all respects similar to that produced by blood.

As might be expected from the preceding experiments, I find that saline solution, to which is added one ten-thousandth part of potassium chloride, makes an excellent circulating fluid in experiments with the detached heart.

On May 31, with the temperature of the room 17·5 C., I first took a tracing with blood mixture, and then replaced this with 100 c. c. saline solution, to which I had added 1 c. c. of 1 per cent. solution of potassium chloride. The contractions continued quite unaltered, and complete contractions still persisted an hour after the heart was first supplied with the saline and potassium mixture. On another occasion, after taking a trace with blood mixture, I substituted for the blood saline solution with 1 c. c. of 1 per cent. solution of potassium chloride solution. The contractions retained the same character, and at the end of four hours and a half were almost as good as at the beginning of the experiment. On another occasion I used sulphate of potash, adding 1 c. c. of 1 per cent. solution to 100 c. c. of saline. This in no way changed the character of the contractions from those occurring with blood mixture, and at the end of four hours and a half the contractions were only very slightly weaker than at the commencement of the experiment.

EXPLANATION OF PLATES.

Fig. 0.

A. Trace with blood mixture.
B. Twelve minutes after saline solution was substituted for blood mixture.
C. Twenty minutes after saline solution substituted for blood mixture.
D. Thirty-three minutes after saline solution was substituted for blood mixture.
E. From another experiment. Thirty-six minutes after saline solution was substituted for blood mixture.
F. Sixty-five minutes after saline solution substituted for blood mixture.
G. See Text.
H. See Text.
I. See Text.

Fig. 0a.

Trace with quicker rate.

A. Trace with blood mixture.
B. Taken about half-an-hour after saline solution was substituted for blood mixture.

Fig. 1.

A. Trace with blood mixture.
B. Trace when saline solution was substituted for blood mixture.
C. Showing partial tetanus by fusion of the beats, owing to great prolongation of the contractions.

Fig. 2.

Showing the effect of blood mixture on the saline trace.

At the point indicated by the arrow 50 c. c. of blood mixture were added to the circulating saline, and the contractions speedily became less frequent and weaker, and of shorter duration. In a short time good normal contractions returned.

Fig. 2a.

A. Trace with blood mixture.
B. Sixty-three minutes after saline solution substituted for blood mixture.
C. About six minutes after saline solution replaced by blood mixture.
D. Forty-two minutes after saline solution was replaced by blood mixture.

Fig. 3.

A. Effect of 5 c. c. of 20 per cent. solution of sodium chloride added to 100 c. c. of blood mixture. Sodium chloride solution added at the place indicated by an arrow.

B. Trace after the addition of 50 c. c. of water to the circulating fluid and the subsequent addition of 2 c. c. of sodium chloride solution 20 per cent.

C and D. After the substitution of saline solution for blood mixture.

Fig. 3a.

Showing the effect of adding a strong solution of sodium chloride to blood mixture.

A. Trace with blood mixture alone.

B. After the addition of 6 c. c. of 10 per cent. solution of sodium chloride.

C. After the addition of 9 c. c. of sodium chloride solution.

D. After the addition of 12 c. c. of sodium chloride solution.

E. After the addition of 14 c. c. of sodium chloride solution.

Fig. 4.

Showing the effect of water on the ventricle and the antagonizing effect of sodium chloride.

A. Tracing with blood mixture.

B. After substituting water for blood mixture. The arrow indicates the time water was substituted for blood.

C. After the addition of 2 c. c. of 20 per cent. solution of sodium chloride. The arrow indicates the time the sodium chloride solution was added to the circulating water.

D. Effect of further addition of 1 c. c. of 20 per cent. solution of sodium chloride.

E. Later effect.

F. Effect of another c. c. of 20 per cent. solution of chloride of sodium, 4 c. c. in all, to the 100 c. c. of water. The trace well shows the effect of sodium chloride solution in prolonging dilatation.

G. Effect of 25 c. c. of white of egg mixture. One part of white of egg to two parts of water.

Fig. 4a.

A. Shows the trace with blood mixture, and the effect of replacing this with 100 c. c. of distilled water, and the effect of adding 3 c. c. of 20 per cent. solution of sodium chloride. The water was substituted at a, and the sodium chloride solution was added at b.

B. Eleven minutes after the addition of sodium chloride.

C. Thirteen minutes after the addition of sodium chloride solution. I then replaced the water and saline by blood mixture.

D. Nine minutes after, substituting blood mixture for the water and saline.

FIG. 5.

Shows the effect of physiological doses on the saline trace.

A. Trace taken with blood mixture.

B. Fifteen minutes after blood mixture was replaced by saline solution.

C. Effect of adding 0·7 c. c. of 1 per cent. solution of potassium chloride to the 100 c. c. of circulating saline solution. The arrow indicates the time the potassium chloride was added.

D. Effect ten minutes later.

FIG. 6.

Shows the effect of physiological doses of potassium chloride on the later stages of the saline solution trace. All the contractions were excited by faradic shocks.

A. Trace with blood mixture.

B. Thirty-three minutes after substituting saline solution for blood mixture.

C. One hundred and ten minutes after substituting saline solution for blood mixture.

D. Immediate effect of adding to the 100 c. c. of circulating saline solution 1 c. c. of 1 per cent. solution of potassium chloride.

E. Eleven minutes after the addition of potassium chloride.

F. Twenty-two minutes after the addition of potassium chloride.

G. Thirty-four minutes after the addition of potassium chloride.

FIG. 7.

Showing the effect of potassium chloride solution.

A. Blood replaced at first arrow by 100 c. c. distilled water containing 5 c. c. of 10 per cent. solution of potassium chloride.

Another c. c. of potassium chloride solution added at second arrow. Two c. c. of potassium chloride solution (10 per cent.) added at third arrow.

B. Shows the effect of substituting blood solution for potassium chloride solution. Blood mixture substituted at arrow.

C. Shows effect of again substituting potassium chloride solution for blood mixture. At first arrow 100 c. c. of water containing 5 c. c. of 10 per cent. solution of potassium solution substituted for blood mixture. At second arrow added 5 c. c. of 10 per cent. solution of potassium chloride. At third arrow substituted saline solution for potassium chloride solution.

D. Shows the effect of 100 c. c. saline containing 2 c. c. of 10 per cent. solution of potassium chloride. This mixture substituted at arrow.

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A FURTHER CONTRIBUTION REGARDING THE INFLUENCE OF THE DIFFERENT CONSTITUENTS OF THE BLOOD ON THE CONTRACTION OF THE HEART.

By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.* (Plate I.)

After the publication of a paper in the Journal of Physiology, Vol. III., No. 5, entitled "Concerning the influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricle," I discovered, that the saline solution which I had used had not been prepared with distilled water, but with pipe water supplied by the New River Water Company. As this water contains minute traces of various inorganic substances, I at once tested the action of saline solution made with distilled water and I found that I did not get the effects described in the paper referred to. It is obvious therefore that the effects I had obtained are due to some of the inorganic constituents of the pipe water.

Water supplied by the New River Water Company contains 278·6 parts of solids per million.

They consist of:

Calcium	38·3	per million.
Magnesium	4·5	„
Sodium	23·3	„
Potassium	7·1	„
Combined Carbonic Acid	78·2	„
Sulphuric Acid	55·8	„
Chlorine	15	„
Silicates	7·1	„
Free Carbonic Acid	54·2	„

This water is faintly alkaline to test-paper from bicarbonate of lime. Saline made with this water I found at first rounds the top of the trace of each contraction and later greatly prolongs diastolic dilatation, and that these effects are completely obviated by about 1 c. c. of 1 °/₀ solution of potassium chloride solution to the 100 c. c. of circulating saline. So that with this addition the saline thus made forms an excellent artificial circulating fluid for the heart, as in some experiments I found the ventricle fed by this mixture would continue beating well for more than four hours, indeed at the end of that time the contractions were almost as good as at the commencement of the experiment, when the ventricle was fed with blood mixture.

In this paper I have investigated the action, on the contraction of the ventricle, of calcium and sodium salts when added to saline solution.

The saline solution contained 0·75 % of sodium chloride.

The lime water I used was prepared from calcined marble and from an analysis by Mr Lawes contained 1 part of CaO in 700 parts of water or 1 part of Ca in 980 parts.

Mr Page kindly prepared some bicarbonate of lime, by diluting lime water with an equal quantity of distilled water and passing carbonic acid through this till the precipitate was completely dissolved, this solution therefore contained half as much lime as lime water.

I used "pure anhydrous" calcium chloride prepared by Hopkins and Williams. My solution contained 1 part of calcium chloride in 390 parts of water or 1 part of Ca in 1082.

I used a 1 % solution of potassium chloride. The salt was obtained of Morson.

The sodium carbonate and the sodium bicarbonate each contained one half per cent. of the salt. The sodium bicarbonate was pure and obtained of Hopkins and Williams.

The distilled water was prepared by Hopkins and Williams.

The distilled water, the saline solution made from it, the calcium chloride solution and the potassium chloride solution were all neutral to delicate test-paper.

The contractions marked in the figures with an asterisk were excited by a break induction shock. Those without the asterisk were spontaneous beats.

These observations were made in October and November.

The traces run from left to right.

These observations were made with Roy's tonometer, and the ventricle was tied on the cannula, as nearly as possible in the auriculo-ventricular groove.

I find that calcium, in the form of lime water, or bicarbonate of lime or chloride of calcium, even in minute doses produces the changes in the ventricular beat described in my former paper. Each of these at first rounds the top, and also broadens the trace of the beat; next it greatly prolongs diastolic dilatation.

Cold greatly favours the action of calcium salts as is well seen by comparing Fig. I. when the temperature was low with Fig. X.

As this slow diastolic dilatation occurs with calcium chloride solution added to saline solution, both neutral solutions, it is obvious that lime possesses the property of retarding dilatation, and that in the case of bicarbonate of lime, the prolongation is not due to alkalinity. The effects produced by lime salts on dilatation, and even the persistent spasm induced by the alkaline preparations, are completely removed by a minute trace of a potassium salt, 1 in 10,000 to 15,000.

A small quantity of calcium bicarbonate or calcium chloride (of chloride 1 in 19,500 parts), added to saline solution with 1 part of potassium chloride in 10,000 parts, makes a good artificial circulating fluid and the ventricle will continue beating perfectly for more than four hours, with calcium bicarbonate.

The solution composed of saline solution, with calcium chloride and potassium chloride is neutral, hence the ventricle will beat perfectly when supplied with a neutral fluid, and therefore alkalinity of the circulating fluid is not necessary for contractility.

The alkaline reaction of the blood is no doubt necessary indirectly, for contractility, for muscular contractions develope acidity and this must give an acid reaction to a neutral fluid, and the heart cannot contract when supplied with an acid circulating fluid.

When the circulating fluid is composed of saline solution the ventricle grows weaker and weaker and contractility ceases in about twenty minutes. Calcium bicarbonate, or calcium chloride in physiological doses, or even in smaller quantities than are present in the blood, restore good contractions, even when contractility has been lost for seven or eight minutes, and the ventricle no longer responds to strong induction shocks.

Sodium bicarbonate added to saline broadens the beat and rounds its summit and slightly prolongs both contraction and dilatation. Owing to the increased breadth of the beat, if the contractions are frequent, there occurs partial fusion of the beats. Sodium bicarbonate also induces some persistent spasm (contracture) if the contractions are frequent.

All of these effects of sodium bicarbonate are obviated by a physiological quantity of potassium chloride.

When a ventricle supplied with saline has lost its contractility, it can be restored for a short time by adding to the 100 c.c. of saline solution 5 c.c. of sodium bicarbonate solution.

On the addition of the potassium chloride solution the effects of the sodium bicarbonate are removed but the contractions quickly grow weak and soon stop; and I have not been able to discover a mixture consisting of saline solution, sodium bicarbonate solution and potassium solution, which will keep the ventricle beating for more than a short time, although I have mixed these solutions in very various proportions.

Calcium chloride solution added to the saline, sodium bicarbonate and potassium chloride solution, after the ventricle has lost contractility, restores good spontaneous beats which will continue for a long time.

A mixture containing 100 c.c. saline, 5 c.c. sodium bicarbonate solution, 5 c.c. calcium chloride solution with 1 c.c. potassium chloride solution also makes an excellent artificial circulating fluid, for with this mixture the heart will continue to beat perfectly.

The heart's contractility cannot be sustained by saline solution nor by saline containing potassium chloride, nor with saline solution containing bicarbonate of soda, nor by saline solution containing bicarbonate of soda and potassium chloride; but after contractility has ceased, the addition of a lime salt will restore good contractility. The addition too of a calcium salt to any of the above solutions will sustain contractility. I conclude therefore that a lime salt is necessary for the maintenance of muscular contractility.

But whilst calcium salts are necessary for the proper contraction of the heart, yet if unantagonized by potassium salts the beats would become so broad and the diastolic dilatation so prolonged that much fusion of the beats would occur and the ventricle would be thrown into a state of tetanus.

As the ventricle will continue to beat perfectly for hours without any sodium bicarbonate, it is evident that the normal trace is the result of the antagonizing action of calcium and potassium salts.

If these two salts are not present in the correct proportions then the trace becomes abnormal. If too little potassium is present, the contractions become broader &c. and there results fusion of the beats. If too much potassium is present, or too little lime salts, then the contraction of the ventricle is imperfect, and by increasing the quantity of potassium salt the beat becomes weaker and weaker till it stops.

As the heart will continue to beat quite normally for hours without albumen or hæmoglobin, it is obvious that these substances are not immediately necessary for contraction, but of course they are necessary to reconstruct the tissues from the loss due to contractions.

The action of simple saline on the ventricle.

Saline is incapable of sustaining the contractions of the heart, for when blood mixture is replaced by saline, the contractions speedily grow weaker, and in some cases contractility ceases altogether, for no contraction can be excited by even a strong break induction shock. In most cases however the beat returns and slowly improves, but rarely becomes so good as with the blood mixture, see Fig. I A and B. Soon the contractions again grow weaker (see C and D), and in about twenty minutes contractility is lost, for the ventricle neither beats spontaneously nor will respond to strong induction shocks. After the contraction is considerably weakened the end of the diastole is a little prolonged (see C), and when the contraction is still further weakened both contraction and dilatation are slightly prolonged (see D), this prolongation being still more marked in cold weather.

Lime water added to saline at first broadens the trace of each contraction and rounds its top, next it excites slight contracture, later the dilatation becomes prolonged. These effects are well seen in Fig. II, in which experiment 0·5 of lime water was added to 100 c. c. of saline. As with saline alone, the strength of the contractions rapidly decreased, but they soon recovered much more rapidly than with saline alone (Fig II A). In about eight minutes the trace became broader and rounder at its top and there occurred a little contracture, see B. Twenty-four minutes after the employment of the saline and lime solution the dilatation of the ventricle became much prolonged and was especially slow at its middle third (see C). 0·5 c. c of 1°/₀ solution of chloride of potassium, rapidly affected the diastole, rendering it as rapid as with blood mixture, and also removed the contracture (see C).

Larger doses of lime water prevent the fall in the trace occurring soon after replacing blood mixture by saline solution, but these larger doses subsequently produce a considerable amount of contracture.

On substituting saline solution for blood mixture, the contractions rapidly weaken and often do not recover or only partially recover and then again grow weak and soon contractility ceases. If when the ventricle has become weak or its contractility has ceased, a small dose of lime water is added to the circulating fluid contraction soon recommences or becomes stronger, as is well seen in Fig. III B where the contractions rapidly increased in height after the addition of 1·1 c. c. of lime water to 200 c. c. of saline.

The effect of lime on the diastole is often earliest witnessed at the end of the dilatation as is well seen in Fig. IV and Fig. V.

Bicarbonate of lime also greatly retards the dilatation of the ventricle. I first took a blood trace and then substituted for the blood mixture 200 c. c. of saline solution containing 20 c. c. of bicarbonate of lime solution. There occurred none of that weakening of the contractions which always ensues with saline solution alone. In about nine minutes after replacing the blood mixture by the saline and lime mixture the dilatation became greatly delayed (Fig. VI). About fifteen minutes after the employment of the saline and carbonate of lime solution, I added to 100 c. c. seven minims of 1°/₀ solution of potassium chloride. This soon quickened diastolic dilatation, but as it still remained somewhat prolonged at its termination, I added another three minims of potassium chloride solution and entirely removed the effect of lime on the dilatation, the trace becoming just like that with blood mixture (see Fig. VI).

I find that from 7·5 c. c. to 10 c. c. of bicarbonate of lime solution to the 100 c. c. saline will prevent the weakening of the contraction occurring when saline solution is alone used.

Is this effect on rhythmic dilatation due to the alkalinity of the calcium hydrate and the calcium bicarbonate or is it due to a property of lime salts? To answer this question I made two sets of experiment. I tested the action of calcium chloride, a neutral preparation of lime, and also the effect of other alkaline salts, as sodium carbonate and bicarbonate. I find that calcium chloride prolongs the dilatation of the ventricle and broadens the trace, rounding its top, but it does not excite any contracture in the small doses I used. To affect the ventricle it must be used in doses considerably larger than lime water. This result is well shown in Fig. VII. I replaced the circulating blood by a mixture containing 100 saline with 3·5 c. c. of calcium chloride solution. The contractions quickly grew weaker but very speedily recovered, so that in about four minutes after the employment of saline and calcium chloride solution the ventricle had recovered. In twenty-four minutes after replacing the blood, the dilatation of the ventricle became prolonged and this prolongation was still more marked eight minutes later. This prolongation was speedily removed by adding to the 100 c. c. circulating fluid 0·33 c. c. of 1°/₀ solution of potassium chloride.

Like calcium hydrate, calcium chloride strengthens the beats weakened or arrested by simple saline solution. This is well seen in Fig. VIII. I first took a trace with blood mixture (A) and then replaced the blood by saline solution. The contractions rapidly grew weak and almost stopped. They then improved somewhat. In eight minutes after the substitution of saline solution I added to 100 c.c. saline 3·5 c.c. of calcium chloride solution. This rapidly increased the strength of the beats (B).

It is obvious therefore that the slow dilatation, and the improvement of contractions caused by lime salts when added to saline solution are not the result of alkalinity, but these effects are due to lime, for the calcium chloride is neutral.

The previous experiments show that calcium oxide, or calcium bicarbonate, or calcium chloride, added to saline solution, prevent the weakening and arrest of the ventricle which occurs with saline solution alone.

They also show that after a ventricle fed with saline solution has become very weak or has stopped, a calcium salt will restore good contractions.

But with calcium salts added to saline the trace of a contraction becomes broader, its summit rounded, and the dilatation is much delayed. All these effects are speedily removed by a physiological quantity of potassium chloride or any potash salt.

I next tried the effect on the trace of substituting for blood mixture saline solution containing a calcium salt and potassium chloride.

I first employed the following mixture, 200 c.c. saline solution containing 20 c.c. calcium bicarbonate solution and 1·5 c.c. potassium chloride solution. On substituting this mixture for blood mixture the beats for a short time grew weaker, but soon recovered and became stronger than with blood mixture. No prolongation of diastolic dilatation occurred, this being obviated by the potassium chloride. The ventricle continued beating perfectly for fifty-six minutes, when I was obliged to discontinue the experiment, see Fig. IX.

I next tested the effect of a mixture composed of 100 c.c. saline, 5 c.c. calcium chloride solution, 0·75 c.c. potassium chloride solution. I first took a trace with blood mixture and then replaced the blood with the above mixture.

The contractions were very little weakened and soon recovered and remained unchanged, no prolongation of the diastole occurring. An hour after the replacement of blood by the saline, lime, and potash mixture the ventricle beat spontaneously, the beats being quite as good as at the commencement of the experiment with blood. See Fig. X.

This solution was quite neutral to test-paper, hence it is evident that it is not necessary for the proper contraction of the ventricle that the circulating fluid should be alkaline.

With saline containing a calcium salt after the diastole becomes retarded the contraction grows weaker, but potassium chloride not only prevents the delay in dilatation but also the weakening of the contractions.

I next investigated whether any other salts added to saline would sustain the contraction of the ventricle.

I tested saline to which I added potassium chloride, 200 c. c. saline with 1·5 c. c. of potassium chloride 1°/₀ solution. As with simple saline the contractions grew weak and then slowly recovered to a considerable extent, and then again grew weak, and in fifteen minutes after replacing the blood mixture with saline and potassium chloride solution the ventricle almost stopped, the trace becoming a mere wavy line. Potassium chloride added to saline therefore does not prevent the ventricle losing its contractibility. After the ventricle had almost completely lost its contractility I added to 100 c. c. of the circulating fluid 5 c. c. of the calcium chloride solution and very speedily spontaneous contractions began which quickly increased in strength.

I next tested the action of sodium carbonate. I employed a 0·5 °/₀ solution. This acts much like sodium hydrate. It rounds the top of the trace, produces persistent spasm and arrests the ventricle in systole. A small dose of potassium chloride, 0·5 c. c. of 1 °/₀ solution antagonizes the effect of sodium carbonate.

I then tested the influence of sodium bicarbonate added to saline solution.

In these experiments I employed 2·5 c. c. of ½ °/₀ solution of sodium bicarbonate in 100 c. c. of saline.

The influence of sodium bicarbonate varies according to the frequency of the contractions. If the ventricle beats infrequently, or not beating spontaneously, it is stimulated only occasionally, then when fed with saline and sodium bicarbonate solution, the beats grow weaker and weaker till they stop, but contractility lasts longer I think than with saline solution alone. When the contractions are much lessened the dilatation becomes rather prolonged, especially at its termination, but the character of the trace is very different from that produced by lime salts.

When the ventricle beats with normal frequency, the contractions on first replacing blood mixture by saline and sodium bicarbonate solution rapidly grow weak, but soon recover and in many instances become as good as with blood mixture. The trace of each contraction becomes broader and its summit rounder, the trace then becomes still broader till partial fusion occurs (see C, Fig. XI), and the whole trace becomes much raised above the base line. There also occurs much persistent spasm. On the addition of 5 min. of 1 °/₀ solution of potassium chloride solution, the effect of the bicarbonate of soda is quite obviated, and the trace falls to its old position as regards the base line, but the contractions are much feebler than at the beginning of the experiment (see C).

If no potash is added to the circulating fluid, but the ventricle throughout is fed with saline and sodium bicarbonate solution, then after a time the beats grow less frequent, so that fusion disappears and the persistent spasm declines; the trace falls to its primary position as regards the base line, and the beats grow smaller and smaller till they cease. If the beats do not become less frequent, the persistent spasm gradually declines, the trace falls again to the base line, though some fusion persists, and the beats become smaller and smaller till they cease.

When a ventricle is not beating spontaneously, contracture can be developed by exciting several contractions quickly one after the other, but after the contractions have ceased, contracture gradually declines, but will again appear on exciting a fresh series of contractions. When contracture has quite subsided, then the dilatation of the first excited contraction is not prolonged, but the dilatation of the succeeding contractions becomes prolonged as the persistent spasm becomes developed, showing that probably the prolongation of the dilatation in this instance is due to the contracture, see Fig. XII.

Sodium bicarbonate will restore contractions to a ventricle that has lost its contractility when fed with saline solution. 2·5 c. c. of the ½ °/₀ solution of sodium bicarbonate added to 100 c. c. of circulating fluid is enough for this purpose. As the contractions return the effect of the sodium bicarbonate becomes manifest, for both contracture and fusion of the beats occur, and after a short time, about seven to twelve minutes, the ventricle stops in partial systole.

As potassium chloride obviates the effect of sodium bicarbonate and prevents contracture and fusion, I determined to try if I could produce an efficient circulating fluid by the addition of some potassium chloride to the saline and bicarbonate solution. I have, however, been unable to do this, though I have added the potassium chloride in various quantities; with any dose that is enough to obviate the effects of the sodium bicarbonate, the contractions become much weakened and soon cease. After the ventricle supplied with saline and bicarbonate solution to which potassium chloride solution has been added, had stopped, I could always restore good contractions by the addition of from 3·5 c. c. to 5 c. c. of calcium chloride solution to the 100 c. c. of circulating fluid, and the contractions became as good as at the commencement of the experiment with blood mixture.

We have seen that a perfect contraction can be obtained with a neutral circulating fluid composed of saline solution with a minute trace of calcium chloride and potassium chloride. Bicarbonate of soda, on which the alkalinity of the blood is supposed to depend, when added to saline is not sufficient to sustain the ventricle's contractility nor even when potassium chloride is added. I next proceeded to learn how a mixture containing saline solution, calcium, chloride and sodium bicarbonate solution affects the ventricle. I find that the sodium bicarbonate appears to exert no influence, and that the whole effect is due to the lime, for after the usual interval and before the ventricle is weakened in any degree, the diastolic dilatation becomes much prolonged, and this prolongation is obviated by a very small dose of potassium chloride, so that good contractions are produced.

In these experiments I used 100 c. c. of saline solution with 3·5 c. c. of calcium chloride solution and 5 c. c. of sodium bicarbonate solution.

On replacing blood mixture with this mixture, the contractions are but little weakened, and only for a short time. The trace of each contraction becomes rounder at its summit, and the dilatation prolonged; but on the addition of 5 minims of 1 °/₀ solution of potassium chloride, the trace becomes of the same character and as good as with blood mixture. With the mixture the spontaneous beats continue, see Fig. XIII.

In the blood therefore sodium bicarbonate must exert a very small influence, if any direct influence on the cardiac contraction, and this is regulated by the antagonizing action of calcium and potassium salts. Still the sodium bicarbonate plays a very important indirect influence, by neutralizing the acid reaction produced by the contraction, for without this neutralizing effect the tissue and blood would soon become acid, when all function becomes impossible; hence sodium bicarbonate and other alkaline salts will prevent these tissues poisoning themselves by producing an acid condition.

I next tested the effect of a solution containing 100 c. c. saline solution, 5 c. c. calcium chloride solution, 2·5 c. c. sodium bicarbonate solution and 0·75 c. c. potassium chloride solution, see Fig. XIV.

I replaced blood mixture by this solution. Very little weakness in the contractions ensued on the replacement and this was speedily recovered from, and the ventricle continued contracting perfectly and spontaneously, ninety minutes after replacing blood mixture by this solution. No rounding of the top, or delay in dilatation occurred, these effects of lime being quite obviated by the potassium chloride.

EXPLANATION OF THE FIGURES, PLATE I.

Fig. I.

A. Trace with blood mixture and showing the effect of replacing the blood mixture by saline solution. The replacement was made at the point indicated by an arrow.

B. Trace eight minutes after replacing blood by saline solution.

C. Trace fourteen minutes after the replacement.

D. Trace eighteen minutes after the replacement.

E. Shows the effect of adding 5 c. c. of calcium chloride solution to 100 c. c. of circulating saline solution. Contractility returned, the beats became after one stimulation spontaneous and owing to the prolongation of diastole the beat fused.

After the beats ceased the diastole is seen to be enormously prolonged. This is well seen after a single contraction; there also occurred some persistent spasm, for the whole trace is raised somewhat above the base line. The calcium chloride was solution added at the arrow.

F. Shows the effect of 3 minims of potassium chloride solution added to the 100 c. c. of circulating fluid. It quickly removed the persistent spasm and brought the trace nearer to the base line and somewhat hastened diastolic dilatation. The potassium chloride was added at the arrow.

G. Shows the effect of nine minims of potassium chloride solution to the 100 c. c. of circulating fluid. The diastolic prolongation is almost entirely removed.

I obtained the same results in another experiment, but only added 2·5 c. c. of calcium chloride solution, and consequently the diastolic dilatation was less prolonged than with double the quantity of calcium chloride.

Fig. II.

A. Showing the effect of substituting 100 c. c. saline containing 0·5 c. c. of lime water.

At the point indicated by an arrow the blood mixture was replaced by the saline and lime mixture.

B. Eight minutes after substituting saline and lime mixture for blood mixture.

C. The trace twenty-four minutes after replacing the blood mixture with saline and lime solution, and also showing the effect of the addition of 0·5 c. c. 1 °/₀ solution of potassium chloride to the 100 c. c. of saline and lime solution. The potassium chloride was added at the point indicated by an arrow.

Fig. III.

A. Trace with blood mixture.

B. Trace seven minutes after replacing the blood mixture with saline solution. At the point indicated by an arrow 0·5 c. c. of lime water was added to the 100 c. c. of circulating saline and the trace rapidly increased in height.

Fig. IV.

A. With blood trace.

B. Effect of 1 c. c. of lime water added to 300 c. c. of saline, twenty-four minutes after replacing blood with the saline and lime solution.

Fig. V.

A. Trace with blood mixture.

B. Trace eighteen minutes after replacing the blood mixture by 200 c. c. of saline with 2 c. c. of calcic chloride solution.

Fig. VI.

A. Trace with blood mixture.

B. Shows the trace about fifteen minutes after replacing the blood mixture by 100 c. c. saline and 10 c. c. of bicarbonate of lime solution. Also shows the effect of 0·5 c. c. of 1°/₀ solution of potassium chloride added or the point indicated by an arrow.

C. Shows the further effect of another three minims of potassium chloride solution.

Fig. VII.

A. Trace with blood mixture.

B. Trace 40 minutes after replacing blood mixture with 100 c. c. saline with 3·5 c. c. of calcium chloride solution. At the point indicated by an arrow 0·33 c. c. of potassium chloride solution was added to the 100 c. c. of saline.

Fig. VIII.

A. Trace with blood mixture and shows the effect of substituting saline for blood mixture. The saline was substituted at the point indicated by an arrow.

B. Shows the effect of 3·5 c. c. of calcium chloride solution. The calcium chloride was added at the arrow.

Fig. IX.

A. Trace with blood mixture, showing the immediate effect of replacing blood mixture by 200 c. c. saline, 20 c. c. calcium bicarbonate solution with 1·5 c. c. potassium chloride solution. Blood replaced at the point indicated by an arrow.

B. Trace eight minutes after the replacement.

C. Trace fifty-six minutes after replacement.

Fig. X.

A. Trace with blood mixture. These contractions were excited by induction break shocks.

B. Trace taken an hour after replacing blood mixture by 100 c. c. saline 5 c. c. of calcium chloride solution and ·75 c. c. potassium chloride. The contractions occurred spontaneously.

Fig. XI.

A. Trace with blood mixture.

B. Sixteen minutes after the substitution of 100 saline containing 2·5 c. c. of $\frac{1}{2}$ °/₀ solution of sodium bicarbonate in place of blood mixture.

C. Eight minutes later. Also showing the effect of adding to the 100 c. c. five minims of 1 °/₀ solution of potassium chloride.

Fig. XII.

Trace showing the effect of contracture on the prolongation of diastolic dilatation.

The first contractions were spontaneous; on their stopping contracture declined. The two following series were excited by break shocks. With the first there is no prolongation of dilatations, but there is some with the second contraction and still more with the third.

Fig. XIII.

A. Effect of replacing the blood mixture by 100 c. c. saline containing 3·5 calcium chloride solution and 2·5 c. c. $\frac{1}{2}$ $^0/_0$ sodium bicarbonate. The blood replaced by the saline &c. solution at the point indicated by an arrow.

B. Trace twenty-four minutes later, showing the prolonged diastole from the effect of the lime and also showing the antagonizing effect of 3 minims of 1$^0/_0$ solution of potassium chloride, which was added at the point indicated by an arrow.

C. Shows the further effect to two additional minims of potassium chloride solution, five in all. This trace was taken, 44 minutes after the blood was replaced by the saline lime and sodium bicarbonate mixture.

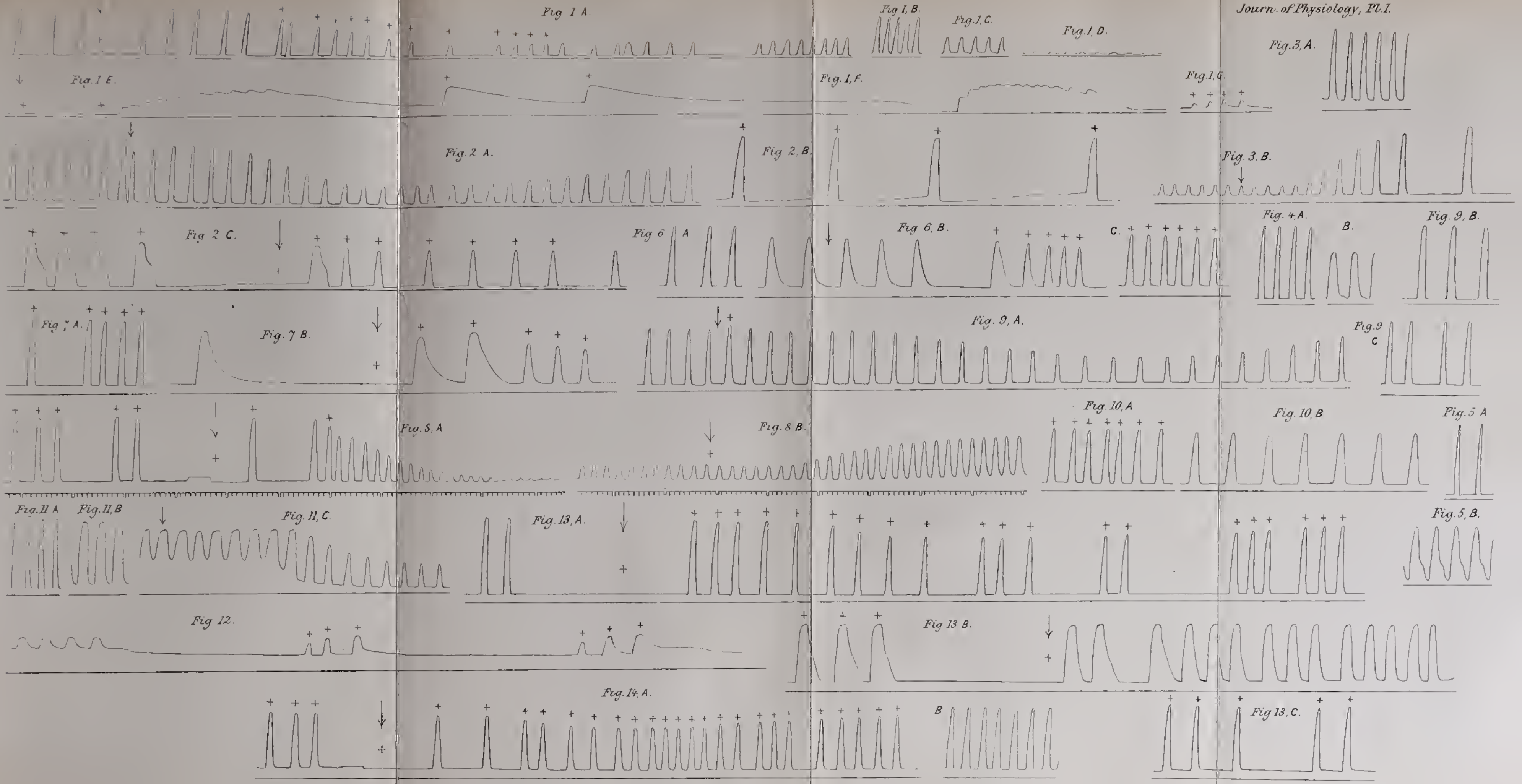
Fig. XIV.

A. Trace with blood, and also shows the effect of replacing blood mixture with 200 saline containing 10 c. c. of calcium chloride solution, 5 c. c. sodium bicarbonate solution ($\frac{1}{2}$ $^0/_0$) and 1·5 c. c. potassium chloride solution (1 $^0/_0$). These beats were excited by induction break shocks. Blood replaced at the arrow.

B. Trace ninety minutes after the replacement.

These beats spontaneous.

Journ. of Physiology, Pl. I.



[*From the Journal of Physiology. Vol. IV. No. 2.*]

A THIRD CONTRIBUTION REGARDING THE INFLUENCE OF THE INORGANIC CONSTITUENTS OF THE BLOOD ON THE VENTRICULAR CONTRACTION.

By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.*

In a previous communication (*Journal of Physiology*, Vol. IV. No. I.) I showed, that without the presence of a lime salt in the circulating fluid, the contractility of the ventricle cannot be supported. I also showed that lime salts greatly delay diastolic dilatation, and that this effect of a physiological quantity of lime is obviated by a physiological quantity of a potash salt.

I further showed that a solution containing sodium chloride and a lime salt only will not sustain contractility so long as a mixture containing a potash salt in addition. For operating with saline solution and lime only, the characteristic lime effects appear, in about half to three quarters of an hour, i.e. the ventricular contractions grow weak and contractility soon ceases; but if in addition to the saline solution containing a calcium salt, a physiological quantity of potassium chloride is superadded, then good contractility will persist for an hour to two hours with only slightly weakened contractions, being about a third less strong.

We see then that a potassium salt by its influence on dilatation is essential to the maintenance of contractility.

I showed too that when fed with a neutral fluid the ventricle will contract well for ninety minutes or longer.

Moreover I demonstrated that sodium bicarbonate added to saline solution will not sustain contractility, nor will saline solution with sodium bicarbonate and potassium chloride. Yet sodium bicarbonate or rather an alkaline state of the fluid does favour contractility as the following experiments show.

A ventricle fed with the neutral solution, 100 c.c. saline, 2·5 c.c. $CaCl^2$ solution 0·5%, and 0·75 c.c. of 1% potassium chloride solution, in the course of an hour to an hour and a half grows weaker, and becomes reduced by one-third its original strength. At this point on the addition of 5 c.c. of a 1% solution of sodium bicarbonate the strength of the contractions becomes notably improved; sometimes indeed the contractions are as good as at the beginning of the experiment.

On another occasion, I replaced blood solution by 100 c.c. saline containing 2 c.c. of 0·5% solution of calcium chloride. The characteristic lime effect on diastolic dilatation ensued. In about forty-five minutes the contractions grew weaker, and when reduced to about one-third their original height I added seven minims of 1% potassium chloride solution. In nine minutes the contractions being still feebler I added 5 c.c. 1% solution of sodium bicarbonate. The spontaneous contractions, which on the addition of potassium chloride stopped, soon recommenced, their strength increased, and thirty-six minutes after adding the sodium salt the spontaneous contractions became equal to the blood mixture standard.

In a similar experiment, extended over a shorter time (about thirty-six minutes), spontaneous contractions restored by sodium bicarbonate became even stronger than with the blood used at the beginning of the experiment.

To what is this improvement due? Is an alkaline solution necessary to the production of muscular contraction? or is the weakening which sodium bicarbonate removes, really due to the developement of acid by the muscular contractions? It can scarcely be maintained that an alkaline fluid is necessary seeing that, though weakened, yet fairly good contractions persist after feeding the ventricle with a neutral solution for ninety minutes and more.

It is more probable I think that the acid developed in the muscles under contraction gradually weakens and at last suspends contractility.

This much at least is certain that the sodium bicarbonate acts by virtue of its alkalinity and not as a soda salt, for the addition to the circulating fluid of calcium hydrate or ammonium carbonate will produce the same restoration of the beats.

I replaced blood solution by 100 c.c. saline containing 2·5 c.c. of 0·5% solution of calcium hydrate. When the contractions became weak I added a small quantity of potassium chloride and the contractions became weaker still. I then added to the 100 c.c. of circulating fluid 1 c.c. of lime water and the contractions quickly improved.

On another occasion I replaced blood mixture by 100 c.c. saline, 2 c.c. calcium chloride ·5%, and 0·75 c.c. potassium chloride 1%. In about twenty-five minutes the contractions were reduced to about $\frac{1}{3}$ their original amount. Then I added 1 c.c. lime water and the contractions quickly improved, and became as good as with blood mixture; subsequently the lime caused much persistent spasm.

Carbonate of ammonia also improves the contractions.

I replaced blood mixture by 100 c.c. saline with 5 c.c. calcium chloride ·5%. In forty minutes the contractions became very weak with marked lime effect. I added 0·75 c.c. of 1% of potassium chloride and the contractions became still weaker. In eight minutes after adding the potassium chloride I added in successive doses 10 c.c. carbonate of ammonia solution ·5%; the contractions improved greatly but did not become quite so good as when operating with blood mixture.

We see then that the amount of contraction, the duration of the contraction, the breadth of the trace, and the rapidity of diastolic dilatation, depend entirely on the relative quantity of the normal saline constituents of the blood.

With calcium chloride added to saline, if the rhythmic contractions retain their normal frequency, we get increased breadth with rounding of the top of the trace, which leads to fusion of the beats and the trace rises high above the base line. A physiological quantity of a potash salt added to the circulating fluid, quickly removes all the lime effects and restores good normal contractions.

If the contractions are less frequent or occur only with an excitation the calcium chloride like other lime salts at first broadens the trace, rounding its top and then greatly retards diastolic dilatation. Potassium chloride obviates these effects.

The retardation of dilatation may be removed, but a little larger dose makes the beat quite normal, sharpening the top of the trace and decreasing its breadth. If a larger quantity is added, the height of the trace becomes reduced. If when the trace is somewhat weakened by potash, 5 c.c. of 1% solution of sodium bicarbonate is added to 100 c.c. of circulating fluid, the contractions regain their full strength but again become broadened and their tops rounded; but this broadening is again removable by a further dose of potassium chloride, which if large enough will again weaken the contraction. The addition of another 2·5 c.c. of 0·5% solution of calcium chloride to the 100 c.c. of circulating fluid will again reproduce complete contractions and the trace may again become broadened; but the addition of more calcium chloride, even 10 c.c. of 0·5% solution, will not prolong diastolic dilatation; in fact with a sufficient quantity of potassium chloride, no quantity of calcium chloride is capable of retarding diastolic dilatation.

[*From the Journal of Physiology. Vol. IV. No.* 6.]

OF THE INFLUENCE OF CERTAIN DRUGS ON THE PERIOD OF DIMINISHED EXCITABILITY. By SYDNEY RINGER, M.D., and HARRINGTON SAINSBURY, M.D. Plate XXI.

In a series of experiments on the comparative effect of certain of the salts of potassium, sodium and ammonium on the activity of the Frog's ventricle, the results of which were given in a paper read before the Medico-Chirurgical Society, June 13, 1882, the effect of continuous faradization applied to the ventricle was described shortly. This effect was much modified by potassium hydrate and potassium salts generally, so much so, and so distinctively that the modification was quite characteristic of these salts.

The effect of continuous faradization applied to the undrugged ventricle is, it will be remembered, to cause what appears to be a blending or fusion of neighbouring contractions. A necessary provision for this is that the intensity of the current and the frequency of interruption be sufficiently great; these being given, the above-mentioned fusion occurs and constitutes continuous spasm. The completeness of this fusion is dependent, caet. par., on the intensity of the current.

Given such fusion, in response to continuous faradization, potassium salts, thrown into the circulation, gradually lessen the fusion and, as the dose increases, remove it completely; finally in the later stages the effect is as follows:—even though at the time spontaneous beats of good value and of good frequency are happening continuous faradization abolishes them completely so long as it is applied; on its cessation spontaneous beats after a longer or shorter interval recur. This result may be reproduced again and again, it is quite definite and constant.

It was toward the elucidation of at least one part of the potash action, viz. its lessening of the above-mentioned fusion, that the present experiments were undertaken. M. Marey[1] it will be remembered applied the term "tetanus" to the fusion resulting from a rapid succession of electric stimuli thrown into the ventricle. The explanation given by him of this phenomenon is involved in the present subject, it may therefore with advantage be restated. Marey, in the first place, shewed that the excitability of the heart was not the same during the whole period of a beat, i.e., during the period comprising both systole and diastole. Thus he demonstrated that a minimal stimulus applied during systole was ineffectual whilst the same applied during diastole was responded to by contraction. The period of diminished excitability he named the "refractory phase," and he shewed that its duration was dependent on the strength of the stimulus applied during the same; the stronger the stimulus, the less the duration, till, with sufficient strength of stimulus the "refractory phase" was completely abolished. Next Marey shewed that a rapid succession of minimal stimuli applied to the heart were responded to by a very much smaller number of contractions, these latter being quite discrete, but that, as the intensity of the stimuli was increased, the rapidity of succession being maintained, the number of contractions increased till finally adjacent contractions overlapped each other and fusion more or less complete obtained. The conclusion drawn was that of the close series of weak (minimal) stimuli the greater number fell during refractory periods and therefore were ineffectual, but that, as with growing strength of stimulus the refractory periods lessened, the number of ineffectual stimuli lessened pari passu, and hence in the final stages the fusion arose. Underlying fusion then we should have, according to this, "diminution" of the refractory period, and the question now to be put is, have we, underlying the potash effect, which shews us the reverse of the above, viz. the undoing of fusion, a "lengthening out" of the refractory period? In addition, the present series of experiments includes the influence of the bases sodium and ammonium; these were examined in like manner to the potassium base.

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[1] *Physiologie Expérimentale*, Vol. II. p. 81, 1876.

Dr Burdon-Sanderson very kindly superintended the arrangement of an apparatus whereby break shocks alone might be used to stimulate the frog's heart. The chronograph was placed in the primary circuit, whilst, by means of a metronome, the pendulum of which carried a wire which was made to dip into a pool of mercury, the primary was short circuited once for every to and fro movement of the pendulum; the moments of make and break were thus recorded by the chronograph. By means of the hammer of the induction apparatus, employed as a magnet key in the secondary circuit, the make shocks were cut off.

Hence the moment of break recorded on the cylinder alone corresponded to the moment of stimulation.

Such an apparatus then records accurately the moment of stimulation, at the same time it allows us, by means of the metronome, to regulate at will the interval between two successive stimuli.

To avoid criticism it may be here stated that the exact measurement of changes in duration was not attempted, the question which it was sought to answer was whether shortening or lengthening of certain periods did occur; this the apparatus here described enabled one definitively to determine.

Since lengthening or shortening, rather than by exactly how much the one or other obtained, was that which concerned us, it was sufficient to note the position of the indicator on the stem instead of taking the actual number of beats as marked on the metronome scale for each position of the indicator, or indeed instead of timing the rate accurately by the watch for each such position. Hence metronome 1, i.e. with the indicator at the topmost mark, indicated the slowest rate, metronome 32 the quickest rate. This range in the positions of the indicator from 1 to 32 gave a considerable range in rate, amounting approximately to a multiplication of the rate by two, or a little in excess of this.

For the rest the apparatus used consisted of a Roy's tonometer and revolving drum. The heart was fed with a mixture of dried bullock's blood dissolved in water and further diluted with two and a half times its volume of saline solution 0·75 per cent. Of this mixture 100 c.c. were used in each experiment.

The common English frog (*R. temporaria*) was throughout employed.

It was important in these experiments that the heart should not beat spontaneously or at any rate that the spontaneous beats should be infrequent, the ligature was hence tied low so as to include part of the ventricle, the upper third where possible.

The experiments were made during the months of June, August and September, 1882.

The mode of experimentation was as follows:—the metronome being set at some given rate, the cylinder was started and two successive stimuli thrown into the heart. The result might be a single contraction or two contractions; if two contractions, these might be quite separate, the diastole of the first contraction being completed before the second systole commenced, or there might be fusion more or less complete. If but one contraction resulted the metronome was set at a slower rate, if two discrete or but partially fused contractions it was set at a quicker rate. In this way the earliest point at which a second stimulus was effective was found. As a rule such earliest stimulation gave fusion complete or all but complete. (See Figs. *A*, *B*, *C*, Trace I.)

An arbitrary but fixed position of the secondary coil was maintained during the above determination.

The length of the "period of diminished excitability"[1] was thus obtained and was seen to comprise:

1. The latent period.
2. A certain portion of the "beat" itself, i.e., of that time during which the heart is undergoing visible change of form.

The position of the second stimulus on the contraction curve enables one to determine the duration of this second element.

Note that the essential in the present mode of experimentation consists in the application of the stimuli in pairs and the ability to regulate at will the length of the interval separating the stimuli of each couple.

The word excitability has in general been employed solely with reference to strength of stimulus; *diminished* and *increased* excitability implying corresponding increase and diminution in the strength of the stimulus adequate to produce a given effect. The use of the term may with advantage be extended and made to refer to the length of the "period of diminished excitability," the strength of the stimulus in this case remaining constant. In this specialized sense the statement that the excitability has been *diminished* will signify that the "period of diminished excitability" has been lengthened out, the reverse, of course, obtaining if the word *increased* be the qualifying term. The repetition of a somewhat lengthy phrase is thus avoided at the same time that there is no likelihood of confusion arising. The word excitability when made to refer to *strength* of stimulus implies a 'prepared' state of the excitable tissue, the stimuli being separated by intervals of sufficient length to permit of recovery from the effects of the one before application of the other; hence an antecedent effective stimulus has no special signification here. When however the word excitability is made to refer to the length of the "period of diminished excitability" the antecedent effective stimulus is an essential element, for the word excitability now refers to an *unprepared state* of the tissue, the result of the preceding activity, and it implies an interval for preparation. If then a longer interval is needed by the heart to recover its excitability, this latter is fitly described as diminished; in like manner if a shorter interval is requisite the excitability may be described as increased. Before experimenting with drugs it was necessary to determine what, if any, variation occurred normally as a result simply of the duration of the experiment and what influence preceding electric stimulation itself had on the excitability as here tested. This being determined it was possible to examine the modifying effect of drugs on the "period of diminished excitability," as a whole, and on its constituent elements corresponding to "Period of Latency" and more or less of the "Period of Active Change."

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[1] The term "period of diminished excitability" is employed in a paper on "the time relations of the excitatory process in the ventricle of the frog's heart," by J. B. Sanderson, M.D., F.R.S., and F. J. M. Page, B.Sc., F.C.S., *Journal of Physiol.* Vol. II. Nos. 5 and 6.

The first experiments on this point shewed that very considerable variation in the "period of diminished excitability" occurred during the experiment, even though this were not much protracted, as a rule not beyond the hour. (It must be remembered that we are throughout dealing with a heart ligatured low.) Thus, to take an example, the metronome would need to be placed say at 1, i.e., at the slowest rate, in order to give the second stimulus effective, at any quicker rate the second stimulus falling within the "refractory period" and being ineffectual; before the end of the experiment it was on several occasions possible to get the second stimulus effective with the metronome at 15. Indeed even with metronome 1 *in*effectual at the start this extent of range was obtained on more than one occasion.

As to the cause of this variation in excitability it soon appeared that whilst, as a rule, an interval of rest would shew diminution in excitability, *given such change*, stimulation would invariably increase the excitability. Thus testing the excitability, then throwing in a number of stimuli in quick succession, and again testing the excitability, this latter was found to have increased; by a repetition of this process it was possible to advance the indicator, division by division, down the scale. This result came out very definitely, clearly establishing as a fact that *stimulation increases the excitability of the ventricle*, i.e. lessens the period of diminished excitability. The same result became apparent in yet other ways, e.g., it has been stated that the ligature was placed low, a portion of the ventricle being almost invariably included within the ligature, still it was not always possible to inhibit the heart, and in some cases groups of beats and intervals of rest would alternate, i.e. phasic action occurred; in these cases the same effect as to excitability was observed to result from these spontaneous beats as occurred with artificial stimulation, viz., the heart after any such phase was found to be more excitable than before it.

Again, the heart would sometimes beat at a sufficiently slow rate to allow of successive stimulation in the intervals, it was then seen that the nearer to the last spontaneous beat one was able to place two successive stimuli, the more excitable did the heart appear, e.g., the metronome at a given figure would just fail to allow of the second stimulus being effective when no care was taken as to the position of the stimuli; when however care was taken to place these as soon as possible after a spontaneous beat, then, without altering the metronome figure, the second stimulus became effective. This result came out very constantly.

Obviously we are dealing here with one and the same phenomenon—if electric stimuli increase the excitability of the cardiac muscle, it is more than likely that natural stimuli will have the same effect, whilst the last point as to proximity to a preceding contraction normally or artificially excited is but a means of demonstrating the phenomenon in question.

Examined further, this phenomenon of lessening of the "period of diminished excitability" was found to be coincident with a lessening of both the "period of latency" and of the "whole duration of the beat"—this result came out unequivocally. The key to the lessening of the "period of diminished excitability" is at once obtained, for this period is relative to the changes latent and visible attending a contraction; if caet. par. both of these changes be shortened in duration the "period of diminished excitability" constituted from both of these must itself be shortened.

Thus then we have as the effect of *stimulation*, lessening of the "period of diminished excitability" as a whole, whilst this effect analyzed into its constituent elements corresponds to a lessening of

1. The Latent Period.
2. The period of active contraction.

It may be asked, does the second element just mentioned, viz., shortening of the *whole* period of visible active change, necessarily involve shortening of the period of diminished excitability; for this *whole* period includes systole and diastole, and shortening of either of these would shorten the whole, whilst the state of diminished excitability stands in relation to the systolic element nearly exclusively. This is true, but the shortening of the duration of the contraction curve resulting from stimulation did not appear to be at the expense of one element more than the other; hence shortening of the *whole* period would involve shortening of the refractory period.

The modifying effect of drugs may now be examined. *Chloride of Potassium* will be first considered. The method of examination was as follows:—the metronome was adjusted to the quickest rate giving the second stimulus uniformly effective, this being found by trial, then an interval of three, four, or five minutes was allowed to pass and the excitability was again tested, the metronome being stationary at the above found figure. Supposing the second stimulus still to be effective this process was repeated, a second, and a third time. Then the drug was added and at the end of a fourth interval of equal duration with the others the excitability was again tested. Thence on through the experiment this method of procedure was maintained. The necessity for this mode of proceeding was that it is impossible to avoid intervals of rest coming in here and there, thus the addition of the drug takes a certain time and then it has to reach the heart, this last with a slow circulation may take from one and a half to two or two and a half minutes, intervals sufficient in some hearts to produce considerable effect, for in some cases the excitability falls quickly on ceasing to stimulate. The above method of examination, in which a definite time interval is fixed on and maintained, obviates the source of error which time introduces, for the kind of change and rate of change due to rest is thus determinable, what remains over and above must be the "Drug effect."

Examined thus it was found that Chloride of Potassium *increased* the "period of diminished excitability"; this result came out uniformly. A moderate dose, it is true, did not always increase this "period" at once, e.g., 0·2 c.c. of a 10 per cent. solution of the salt, and in one or two instances this dose did not produce the effect at all. An increased dose, e.g., 0·4 c.c. of the same solution, given at once, always brought the effect out speedily and strongly.

Examined further, it was found that the "period of Latency" was always increased as an effect of the potassium salt, and further that, as a rule, the duration of the period of visible change, i.e., the breadth of the contraction curve, was shortened.

It is obvious that these two results are antagonistic so far as the "whole period of diminished excitability" is concerned, for, whilst the increased period of latency will, caet. par., pitch the second stimulus early on the contraction curve, the diminished duration of the contraction itself will have an opposite effect. In the slighter degrees of toxic action, it may be one would need to consider both of these elements in the result obtained, but when a well-marked toxic effect has been produced one has no need to balance the above two in order to determine whether or no the diminution in excitability is apparent or real; that it is real, and that we have to deal with a diminished excitability of the cardiac tissue which can not be explained by the position of the second stimulus on the contraction curve is certain, for we may increase the interval between the stimuli to the extent even of throwing the second stimulus after completion of diastole and yet this stimulus remains ineffective. See Figs. *A*, *B*, *C*, *D*, *E*, *F*, Trace II.

Potassium chloride then increases the "period of diminished excitability" as a whole, and it does so by increasing both constituents of this period, viz.,

1. The Period of Latency.
2. The Refractory period attending the contraction.

This latter element, from occupying a certain portion of the contraction curve as it does in the normal, comes to occupy more and more of the curve till finally it may stretch out beyond the beat to a greater or less extent. Together with these changes there goes as a rule diminution in the duration of the beat, though in some cases this effect is apparent rather than real and due to the apex of the beat having become more peaked, the base however remaining of the same breadth. With these changes there is of course a gradual fall in the height of the curve.

Another point which comes out constantly with potassium chloride is that, supposing one starts with a metronome rate giving complete fusion of the two beats, the first effect of the salt will be to lessen this fusion, a dip appearing between the two crests, then the valley deepens, till finally you may get two quite discrete beats; the next effect is that the second beat disappears; all the while the metronome rate has been fixed.

The above effects may be readily summed up in the statement that potassium chloride lessens excitability from the very commencement. For, to enumerate: a given strength of stimulus takes longer to manifest itself, "increased period of latency"; when it does manifest itself, the manifestation is a diminished one, "lessened height and duration of the beat"; the refractory state, normally but slightly involving the period of diastole, now, under the potassium salt, comes to implicate the whole period of the beat or even to extend beyond this; all these are surely but expressions of the above general statement that Potassium lessens excitability. To these may be added the effect noted in former experiments that, as the toxic effect deepens, e.g. in the stage after inhibition, the strength of the stimulus, to be effective, must be increased. There yet remains a somewhat remarkable phenomenon which must not be omitted, and which apparently is witnessed at all stages of the potassium effect, it is that, whether the diminution in excitability effected by the drug be small or great, the effect of stimulation appears to deepen the effect, not to antagonize it, the latent period increasing, the height and duration of the beats growing smaller, the period of diminished excitability lengthening out; thus, a complete reversal of the effect noted in the case of the undrugged heart. Have we not herein some clue to the reading of the phenomenon mentioned at the outset? It is noted, viz., that continuous faradization not only lost its power to produce fusion as the dose of potash was increased, but that in the final stages a reverse effect was observed, viz., the actual suppression of spontaneous beats during the time of faradization. Though perhaps no explanation of the latter, the above facts appear at any rate to be of the same order; both indicating that stimulation would seem to develop or intensify the potash effect, the tissue actually becoming less excitable.

The following experiments were made with a view of determining this point:—Into the secondary circuit of the arrangement which has been described as here employed the two terminals from the secondary coil of another du Bois Reymond induction apparatus, set for faradization, were brought. It was possible now to faradize the heart for a short interval between the two successive break shocks of the original apparatus. For explanation we may refer to any of the diagrams; the process consisted simply in faradizing the heart for a shorter or longer period between the stimuli a_1, a_2 of each couple. It was not possible to begin the faradization directly after a_1 or to continue it directly up to a_2 for fear of touching these boundary limits and so short circuiting the heart, so that the middle period between a_1 and a_2 was the period of faradization. Having then found an effective couple by the ordinary method a series of such couples was taken, but for every alternate couple the heart was faradized between the stimuli. These faradic shocks of course fell during the refractory period and the question to be determined was whether the already existing state of lessened excitability would be intensified by such faradization or uninfluenced; if the former then each faradized couple should shew either a diminution in the fusion of the two beats or a suppression of the second beat. The results obtained were negative, the faradization appeared to be without effect.

This point was however still further tested—the same apparatus was kept—the drug was added and pushed till a marked effect had been produced, till in fact powerful faradic stimulation continuously applied gave but a single initial contraction instead of a rapid succession of beats blending by their proximity such as obtains for the undrugged heart. On this stage of *lowered* excitability it was possible to advance yet a step further, for by repeatedly faradizing, allowing only very short intervals for rest, it was possible even to abolish this initial beat, i.e. the rapid succession of shocks were without effect; when this stage had been reached and immediately after such an ineffectual series, a single break shock was thrown into the ventricle and this invariably gave a contraction. As to the relative strength of the shocks, two separate induction apparatus being employed, the coils were tested for us for given positions, and the secondary coil used for obtaining break shocks only was then moved so as to give decidedly weaker shocks than the coil of the apparatus used for faradizing (we refer here only to the opening shocks of the faradic series). The results then are these—a series of shocks give no effect, a single weaker shock gives an effect; it must then be that shocks falling during a certain part or during all of the period of latency tend to inhibit or interfere with the initial shock of the series. This of course applies only to the frog's ventricle under the influence of a potassium salt.

We may now pass on to consider the other bases.

Ammonium chloride similarly tested gave very different results; here it was necessary to distinguish two stages, a primary and a secondary. The primary stage occurred early in the action of the drug, indeed it set in almost as soon as the drug had reached the heart, its duration however was but short.

During this primary and passing stage the effect on the period of diminished excitability was a variable one; in some cases it was lessened, in others slightly increased. On the period of latency the effect was probably always one of diminution; in some of the cases in which before the addition of the drug, the period of latency was but short, no noticeable diminution could be made out, certainly there was no lengthening. In others in which a very well-marked latent period existed, slight but undoubted shortening obtained; it is more than probable that more delicate means would have detected the same in the previous cases just mentioned. In addition this primary stage was marked by increase in the height of the contraction and increased duration of the beat with broadening of the apex of the beat; the effect on duration was the one chiefly marked. See Figs. *A'*, *B'*, Trace III.

This primary stage, then, with its diminished period of latency and its increased height and breadth of trace, contrasts very markedly with the potassium effect, which on all these points shews the exact reverse.

The effect on the period of diminished excitability has been stated to have been inconstant, the variation either way was however in no case considerable; and since the shortening of the period of latency would have a tendency the opposite of that due to the increased duration of the beat, it seemed not improbable that the predominance of the one effect over the other might account for the variation observed.

So far the primary stage has alone been considered, this however was transitory, gradually the secondary stage developed which, shortly described, presented exactly the potassium effect, the period of diminished excitability as a whole being greatly lengthened out, the latent period being also much increased and the duration of the beat diminished. Here too, as for potassium, the phase of diminished excitability from occupying but a limited portion of the contraction curve, as in the normal, came finally to involve the whole of the curve, both systole and diastole. See Figs. *C*, *D*, *E*, *F*, Trace III.

Sodium Chloride. The dosage with this salt had to be increased according to the approximate toxic ratio already determined in previous experiments, the doses employed were, viz., ten times as large as for the two preceding salts. As to the effects they are not quite so unequivocal as for the potassium and ammonium salts.

The difficulties with respect to the period of diminished excitability were these:—if, subsequently to the addition of the drug, one threw in a series of double stimuli, such as before the dosage had given both stimuli effective, the trace obtained might appear as if the result of a single contraction only; if however an occasional single stimulus were thrown in, comparison of the resulting contraction with that obtained by double stimulation would shew the latter to be both higher and of longer duration. Had one here to do with a case of blending or not? If of blending the fusion was complete, there being not the slightest break in the curve to indicate its composite nature, see Fig. *B*, Trace IV. However that this apparently simple trace is really compound is more than probable, for we get transitional forms from such to a trace which is quite obviously double, see Fig. *B′* and Fig. *C*.

The question of fusion or not is at best of no great importance for it does not touch the point at issue, viz., the effectiveness of the second stimulus, for whether this second stimulus shew itself by causing an obvious second contraction or simply cause the line of systole to rise higher and prolong the period of active contraction, in either case the second beat *is* effective. Moreover it is effective after a shorter interval of time as Fig. *C*, Trace IV. shews.

The latent period is distinctly increased by the sodium salt, making all allowance for the more gradual rise in the line of systole which renders it somewhat difficult to determine exactly when the systole does commence (see Trace IV.).

The effect on the duration of the beat was not very decided; as a rule there was shortening till in the later stages, when the beats became lengthened out, sometimes considerably.

We have seen in the case of the potassium salt and also in the second stage with the ammonium salt that the refractory state gradually encroaches on the line of diastole till finally the whole curve may be involved; if now we compare Fig. *A* with Fig. *C*, Trace IV., we shall note in this latter how early the second stimulus falls as compared with the former, in fact the reverse condition obtains here; under the influence of the sodium salt the refractory state has come to occupy less and less of the period of active contraction. Yet another point of contrast may be instanced; under the potassium salt we have seen that repeated stimulation, instead of increasing the excitability as it does for the undrugged heart, always lowers it; under the sodium salt the influence of stimulation is to increase the excitability. Thus, after a large dose of the sodium salt the first contraction may be very small and the latent period very long; as stimulation is repeated the beats grow in height and the latent period shortens.

The results obtained may be briefly summed up as follows:

In the case of the undrugged ventricle, stimulation shortens the period of diminished excitability; analyzed, this effect shews shortening of the period of latency and likewise of the duration of the period of active contraction.

Passing to the drugged heart we find, that *Potassium chloride* increases markedly the period of diminished excitability; that the period of latency is also considerably increased; that the duration of the beat is as a rule diminished; but that in spite of this the refractory state encroaches more and more on the period of diastole till finally the whole of the contraction curve may be involved and indeed beyond this to greater or less extent; that stimulation appears to intensify the potassium effect, diminishing the excitability still further, the reverse effect to that obtaining with the undrugged heart.

That *Ammonium chloride* shews two stages, a primary stage corresponding to increased height and breadth of beat, here the period of latency is *diminished*, but the period of diminished excitability variously affected, in some cases slightly lessened, in some slightly increased; a secondary stage in which effects similar to those of potassium obtain, viz. increase of the period of latency and of the period of diminished excitability with diminished duration of the beat.

That *Sodium chloride*, when the drug effect is pronounced, shews marked diminution of the period of diminished excitability, and this in spite of prolongation of the latent period; that the duration of the beat is variously affected, except in the final stages when it is lengthened out; that the refractory state hence comes to occupy less of the period of active contraction; finally that repeated stimulation increases the excitability as it does for the undrugged heart.

These results have been given at length, not because they represent the individual effects of the three drugs, potassium, sodium and ammonium chloride, but because they represent probably the effect of potassium, sodium and ammonium salts generally. (For the reasons for this statement see the results of previous experiments recorded in the *Medico-Chir. Trans.* loc. cit. and in the *Practitioner*, Aug. 1882.) As class effects they would naturally possess the greater value. We know from the teachings of physiology that during the excitatory process which results from stimulation of a contractile tissue, this tissue for the greater part of the duration of this process is relatively insusceptible of responding to further stimulation[1]. The interest of the present paper centres around the modification of this refractory state which certain drugs are capable of effecting, which modification is both marked and definite for the particular drug taken. The striking contrast which obtains between the potassium and the sodium salt with respect to this modification and indeed with respect to nearly every point mentioned in relation to this subject, is of the greatest interest, not only because, from a chemical point of view, such contrast would be quite unlooked for in two elements apparently so nearly akin, but also because we are here dealing with fundamental properties of tissues and also because both these elements are important constituents of the animal organism. It can scarcely be doubted but that such marked differences as here appear between the sodium and the potassium salts must indicate their subservience in the organism of functions proportionally distinct in their nature. The strength of this conclusion is scarcely weakened by the fact that we have throughout been dealing with toxic doses.

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[1] With reference specially to the Frog's heart consult the previously mentioned paper by Dr Sanderson and Mr Page, *Journ. of Phys.* Vol. II. Nos. 5 and 6.

In conclusion it may be stated that the results obtained with the potassium salt are compatible with Marey's explanation of the mode of action of a succession of stimuli in producing continuous spasm of the ventricle.

EXPLANATION OF PLATE XXI.

TRACE I. Figs. *A*, *B*, *C* illustrate simply the effect of double stimulation. In *A*, the two stimuli, thrown in at the points a_1 a_2, cause but a single contraction—the second stimulus a_2 falls therefore too early, i.e., during the refractory period.

In *B*, the two stimuli have each had effect, the two beats are however quite discrete.

In *C*, two tracings are given, II. shews the effect of double stimulation with both stimuli effective; fusion is here all but complete. I. is the result of a single stimulus, it is for the sake of comparison.

TRACE II. shews the modifying effect of the potassium salt.

In *A* both stimuli are seen to be effective; note the period of latency and the position of the second stimulus, a_2, on the contraction curve.

In *B* the like is seen; this trace was taken after the heart had been allowed a rest of five minutes.

During the five minutes rest following trace *B*, the potassium salt was added, 0·4 c.c. of 10 per cent. potassium chloride solution. *C*, *D*, *E*, *F* shew the tracings now taken.

Therefore *A* and *B* are before the addition of potassium salt.
C, *D*, and *E* after ,, ,,

The metronome stood at 22 in *A*, *B*, and *C*, in *D* it was pushed up to 5, in *E* to 1. Note the enormous lengthening out of the latent period; the great variation in the refractory period, from metronome 22 to 1, the position of the second stimulus in *E*, where alone it becomes effective; note also that the second stimulus when it does become effective does not yield fusion.

TRACE III. shews the ammonium effect in its early stage.

A is before the addition of the drug.

B subsequent to the addition of 0·3 c.c. of a 10 per cent. solution of ammonium chloride.

A single spontaneous beat is in each case given for comparison with the effects of double stimulation.

Note in *B* the greatly increased duration of the beat, and the greater completeness of the fusion; the effect on the latent period is not marked, but if in any direction it is in that of diminution.

Note that the increased duration of the beat, resulting from the ammonium chloride, tends to pitch the second stimulus, a_2, earlier on the contraction curve.

The later stage of ammonium chloride action is not shewn, it is similar to that of the potassium salt but less pronounced.

In both *A* and *B* the metronome stood at the same figure, viz., 20.

Figs. *A′*, *B′* illustrate again the primary stage of ammonium effect (metronome at 25 in either case), *B′* being subsequent to the addition of the drug 0·3 c.c. of NH_4Cl 10 per cent., the shortening of the period of latency is here well marked.

Figs. *C*, *D*, *E*, *F* shew both stages; *D* shews the primary stage after 0·3 c.c. of NH_4Cl 10 per cent.; *E* and *F* the secondary stage, compare potassium; the metronome in *C* and *D* stood at 15, in *E* it stood at 10, in *F* at 5. The secondary stage will be seen to be exactly like that for potassium chloride.

TRACE IV. shews the sodium effect.

In *A*, I. and II., we have the effect before addition of the drug; between I. and II. the heart had an interval of three minutes rest.

B, *B′*, and *C* are subsequent to the addition of chloride of sodium; between *A*, II., and *B*, in the interval of three minutes allowed, 3 c.c. of a 20 per cent. solution of sodium chloride were added. Note the effect on the period of latency. In *B* two of the curves are the result of double stimulation, two of single stimulation, but for this comparison, the double stimulation here would appear to yield but a single contraction, whilst in all probability there is fusion here, i.e., both stimuli are effective (compare *C*).

In *A*, *B*, and *B′* the metronome stood at the same figure, viz., 15. *B′* was taken after 2 c.c. more had been added, in all 5 c.c. of the 20 per cent. solution, the trace from double stimulation here shewn is distinctly compound.

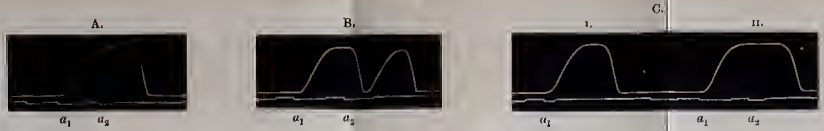
In *C* we have the effect some 15—20 minutes later, in all 5 c.c. of the 20 per cent. NaCl solution had been added. The metronome stood at 27.

Note therefore that the refractory period has grown less, for the curve from double stimulation is obviously double. Double and single stimuli alternate here for comparison.

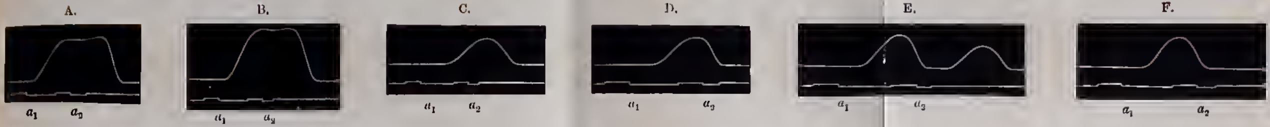
Note how early the second stimulus a_2 falls on the curve and compare with the potassium trace, in which the second stimulus falls low down on the diastole curve before it is effective.

Plate XXI.

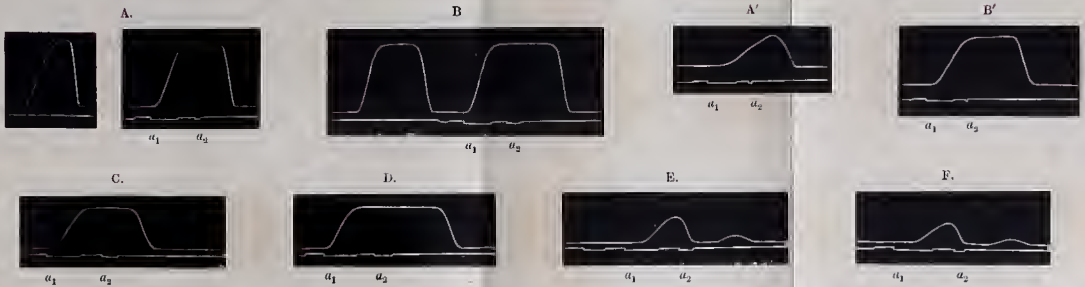
TRACE I.



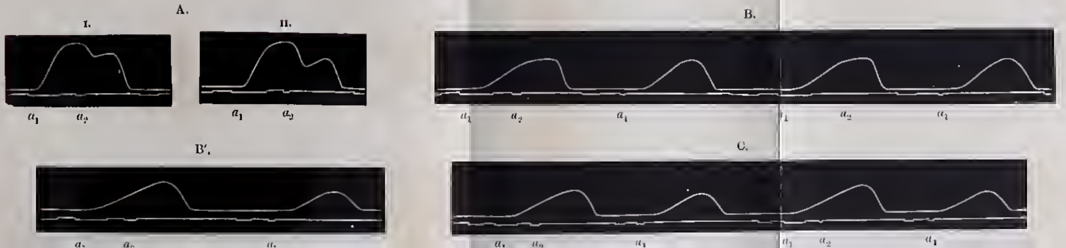
TRACE II. Potassium effect.



TRACE III. Ammonium effect.



TRACE IV. Sodium effect.



[*From the Journal of Physiology. Vol. IV. No. 6.*]

AN INVESTIGATION REGARDING THE ACTION OF RUBIDIUM AND CÆSIUM SALTS COMPARED WITH THE ACTION OF POTASSIUM SALTS ON THE VENTRICLE OF THE FROG'S HEART. By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.* Plate XXII.

It is generally held that chemical similarity indicates similarity in physiological and therapeutic action, but whilst this inference is to some extent true, yet some substances closely allied chemically differ from each other widely in physiological and therapeutic effect. For instance, the physiological action of potash salts differs greatly from the action of sodium salts; and it is well known that, therapeutically, chlorides cannot replace iodides nor either of these take the place of bromides. To further test the correspondence between chemical and physiological action I made some experiments with chloride of calcium, chloride of strontium and chloride of barium[1]. I have shown[2] that a lime salt in the circulation is necessary to maintain the heart's contractility, but that when lime only is added to the circulating saline solution (sodium chloride ·75 p.c.), the diastolic dilatation is greatly prolonged. The addition of a physiological quantity of a potassium salt will obviate this effect, and if to saline solution we add a suitable quantity of a lime and a potash salt and render the solution slightly alkaline with bicarbonate of soda we get a circulating fluid fit to maintain rhythmic contractions in the detached ventricle for many hours. Now I find that in all these respects strontium acts very similarly to lime, that it can indeed to a large extent replace lime. On the other hand barium differs almost entirely from either calcium or strontium in its physiological action. Now these three elements form a group of which strontium is chemically more allied to lime than is barium. The physiological action therefore of these three substances corresponds to a great extent to what we should anticipate; except that we should expect barium to possess more of the characteristic effects of calcium and barium than is actually the case.

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[1] *Practitioner*, Aug. 1883.

[2] *Journal of Physiology*, Vol. IV. Nos. 1 & 2.

The close chemical relationship between potassium, rubidium and cæsium, led me next to investigate the degree of physiological correspondence between rubidium and cæsium on the one hand and potassium on the other.

I employed the chloride of each of these elements, using a solution prepared and calculated on their molecular weights. The potassium chloride solution was ·745 per cent., the rubidium chloride 1·205 per cent. and the cæsium chloride 1·68 per cent.

The experiments were made with the ventricle of the frog's heart attached to a perfusion cannula by a ligature placed as nearly as possible in the auriculo-ventricular groove. An artificial circulation was maintained through the ventricle by a syphon action. I employed Roy's tonometer to record as usual on a revolving cylinder the movements of the lever produced by the contraction of the ventricle. The experiments were made in June and July.

It is necessary to preface these experiments with rubidium and cæsium, with a brief statement of the physiological action of potassium on the frog's heart.

When the circulating fluid supplied to the detached ventricle consists of simple saline solution, the contractility at first grows weak or ceases and then gradually recovers itself more or less completely. Now a physiological dose of potassium chloride prevents the occurrence of this recovery.

When the ventricle is fed with saline solution containing a physiological quantity of a lime salt, diastolic dilatation is much retarded, so that if the contractions remain of normal frequency much fusion ensues. A physiological dose of a potassium salt accelerates this dilatation and makes the contraction similar to that which occurs when the circulating fluid consists of blood.

Sodium carbonate and sodium bicarbonate added to saline solution broaden the beat, cause much fusion of the beats and induce tonic contraction. A physiological dose of potassium chloride obviates all these effects.

When the circulating fluid consists of blood mixture[1] potassium salts

(1) greatly prolong the latent period,

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[1] Blood mixture is made by dissolving dried bullocks' blood so as to imitate fresh blood and diluting this with two parts of saline made with tap water.

(2) greatly prolong the period of diminished excitability and consequently greatly diminish the frequency of the spontaneous contractions and greatly diminish the frequency with which contractions can be excited by electric stimulation.

With blood mixture[1], faradization induces tetanus by fusion of the beats, but when a potassium salt is added to the blood mixture then faradization completely arrests the contractions, which recommence on discontinuing faradization.

Potassium salts arrest the ventricle in diastole.

The following experiments with Rubidium Chloride show that in all the foregoing particulars the action of rubidium corresponds to the action of potassium.

Like potassium chloride, rubidium chloride prevents that recovery of the ventricle which occurs when it is supplied with simple saline solution. With simple saline at first there is considerable weakening of the beats and even arrest of the contractility but considerable or complete recovery soon sets in. On replacing blood mixture with saline solution containing rubidium chloride in the proportion of 1 c.c. of 1·205 per cent. solution of rubidium chloride in 100 c.c. of saline solution the ventricular contractility grows less and less till it disappears. On the addition however of either calcium chloride or strontium chloride good contractions speedily return.

Like potassium, rubidium obviates the effect of a calcium salt on the ventricular dilatation. As I have formerly shown a lime salt added to saline broadens the beat, rounds its top and later greatly retards dilatation. 1 c.c. of 1·2 per cent. solution of rubidium chloride to the 100 c.c. of circulating fluid completely removes these lime effects and restores to the beat the same character it possessed when fed with blood mixture.

Like potassium, rubidium obviates the effect of sodium bicarbonate. I replaced blood mixture by 200 c.c. of saline solution containing 5 c.c. of 1 per cent. solution of sodium bicarbonate; the sodium bicarbonate as usual broadened the beat and caused much fusion of the beats and induced a little tonic contraction. 1 c.c. of 1·205 per cent. solution of rubidium chloride obviated all these effects.

I have elsewhere[2] shown, that a circulating fluid, to sustain the contractility of the heart must be alkaline in reaction and must in addition to sodium chloride contain both lime and potassium salts. The following experiment shows that rubidium chloride will replace potassium chloride in this solution and will sustain the heart's contractility. I replaced blood mixture by 200 c.c. saline solution containing 2 c.c. of 1 per cent. solution of calcium chloride, 1·5 c.c. of 1·205 solution of rubidium chloride and 5 c.c. of 1 per cent. solution of sodium bicarbonate; fifty-five minutes after the substitution the contractions continued in all respects the same as with blood mixture.

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[1] Blood mixture is made by dissolving dried bullocks' blood so as to imitate fresh blood and diluting this with two parts of saline made with tap water.

[2] This Journal, *loc. cit.*

On another occasion after replacing blood mixture by a similar solution, at the expiration of an hour after the substitution, the beats continued as good as the beats with blood mixture.

We find then that rubidium can largely replace potassium and I have shown in a previous paper[1] that strontium can replace lime. I therefore experimented to ascertain whether I could devise a fluid capable of sustaining the heart's contractility containing rubidium and strontium in place of potassium and calcium. Among other combinations I employed the following: 200 c.c. of saline solution containing 2·5 c.c. of 1·58 per cent. solution of strontium chloride, 1·5 c.c. of 1·2 per cent. solution of rubidium chloride and 4 c.c. of 1 per cent. solution of sodium bicarbonate. The character of the contractions remained unchanged but the beat grew slowly weaker so that in thirty-five minutes there occurred a reduction of one third the height of the trace of each contraction. Strontium and rubidium therefore, though decidedly inferior to calcium and potassium, will sustain the contractility for a considerable time.

Dr Sainsbury kindly tested the effect of rubidium on tetanus induced by continuous faradization. Potassium salts, it will be remembered, not merely prevent tetanus by continuous faradization, but so influence the cardiac muscular tissue, that on the application of faradization all spontaneous contractions cease and the ventricle remains in diastole, but on suspending the application of the faradic current spontaneous contractions return. Rubidium in this respect is similar to potassium. At the commencement of the experiment, when blood mixture was alone used he obtained well-marked fusion with the secondary coil at 8. After the addition of 6 c.c. of 1·205 per cent. solution of rubidium chloride to the 100 c.c. of circulating fluid, the fusion became notably less, there was indeed no fusion at all, simply acceleration during the time of faradization; after 10 c.c., the already existing rhythm remained undisturbed, the faradization producing no effect. After 12 c.c. the same obtained; but although the faradization with the coil at 8 had lost all power to cause fusion, there was no actual inhibition of the beats during faradization. On putting the secondary coil at 6, the characteristic potash effect at once came out, viz. the beats ceased completely during faradization, commencing starting again almost at once on its cessation; the same obtained with the coil at 4. Hence increasing the strength of the faradic current, instead of tending towards the production of tetanus, gave an opposite effect.

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[1] *Practitioner, loc. cit.*

Again rubidium like potassium increases "the period of diminished excitability." In these experiments break shocks alone were employed and by means of a metronome placed in the primary circuit, which latter included the time marker, he was able to increase or diminish the interval between successive stimuli and also to record the moment of stimulation. The result showed no very decided effect on the latent period, but if anything the effect was in the way of prolongation: on the period of diminished excitability however the effect was decided. Here the experiment started with the stimuli so distanced as to yield considerable fusion of the two beats; i.e. the pair of stimuli produced a pair of beats which overlapped each other. This occurred with the metronome standing at 22 on the scale, and the secondary coil at 7. After the addition of rubidium chloride, at the end of the experiment the secondary coil at 7 was unable to excite a contraction at all, and with the coil pushed up to 3, it was no longer possible to produce double excitation, except by increasing considerably the interval between the stimuli, viz. with the metronome advanced from 22 on the scale to 5.

Like potassium chloride, rubidium chloride arrests the ventricle in diastole.

Whilst rubidium is so closely similar to potassium, the case is far different with cæsium, the physiological action of this metal being much more akin to barium and strontium.

On replacing blood mixture by 200 c.c. of saline solution containing 1·2 to 2 c.c. of 1·68 per cent. solution of cæsium chloride, the frequency of the spontaneous contractions is much increased, the trace of each beat becomes much broadened with much fusion of the contractions. Much tonic contraction also occurs. The primary fall with simple saline only is prevented or becomes very slight, but cæsium cannot sustain the heart's contractility and so the trace falls nearer and nearer to the base line, and in about twenty minutes all contractility ceases. When the ventricle beats less frequently the trace of each beat then becomes still broader.

The following experiment well illustrates many of the properties of cæsium.

I first took a trace with blood mixture (see Fig. 1. A.). I then substituted for the blood mixture, 200 c.c. of saline solution, containing 2 c.c. of 1·685 per cent. solution of cæsium chloride. The trace of each beat soon grew broader, and tonic contraction ensued. Ten minutes after the change of fluid the trace beat had become very greatly broadened (see B.). The ventricle then grew weaker and weaker till at last contraction almost ceased, when I added 3 c.c. of 1 per cent. solution of sodium bicarbonate to 150 c.c. of circulating fluid. The contractions grew stronger but retained the same character and never became so complete as with blood mixture (see C, ten minutes after adding the sodium bicarbonate).

I then added 1 c.c. of 1 per cent. solution of potassium chloride to 150 c.c. of circulating fluid. This weakened the contractions almost to the point of extinction. On adding 1·5 c.c. of 1·586 per cent. solution of strontium chloride, an equivalent quantity to the cæsium chloride contained in the fluid, the contractions immediately improved and became as complete as with blood mixture, but retained the cæsium quality, indeed they became broader than under cæsium and saline alone (see D. after a long diastolic pause). The length of the contraction was proportionate to the interval of rest between each beat.

I then added 1·5 c.c. of 1·11 per cent. solution of calcium chloride or an equivalent quantity molecule for molecule to the cæsium chloride contained in the fluid and immediately the lime prevailed over the cæsium, the beats grew shorter and shorter till they became almost identical with the beats under blood mixture (see E.). Here whilst an equal molecular quantity of strontium failed to remove the cæsium effects an equal molecular quantity of calcium chloride almost entirely replaced the cæsium effects.

It appears then, that cæsium differs considerably from potassium and rubidium and is closely allied in its physiological action on the ventricle to barium.

Whilst strontium is powerless to antagonize an equal molecular quantity of cæsium, yet by increasing the quantity of strontium the cæsium effect may be overcome.

In the preceding experiment lime antagonizes cæsium but at the same time cæsium antagonizes some of the calcium effects, if the salts are employed in equal molecular quantities.

I replaced blood mixture by 200 c.c. saline solution containing 4 c.c. of 1 per cent. solution of sodium bicarbonate and 2 c.c. of 1·11 per cent. solution of calcium chloride and 2 c.c. of 1·685 per cent. of cæsium chloride. The trace of each beat grew somewhat broader and its top rounded, but no other change occurred. The diastole did not become delayed, nor did any contraction or fusion of the beats occur. The ventricle continued to beat spontaneously and three hours after the substitution, the trace of each contraction measured about one-eighth less and was a little broader and rounder at its top than at the beginning of the experiment with blood mixture.

Here, then, whilst calcium antagonized the cæsium, the cæsium antagonized the calcium preventing its effect on dilatation and in this respect acting like potassium.

Here we have an instance of mutual antagonism. The lime prevented the cæsium effects whilst the cæsium obviated one of the calcium effects and as the result of this mutual antagonism we obtained a good circulating fluid capable of sustaining good contractions for upwards of three hours, the character of the contractions differing little from the contractions with blood mixture.

This mutual antagonism is shown in another interesting way.

I replaced blood mixture by 200 c.c. of saline solution containing 4 c.c of 1 per cent. solution of sodium bicarbonate and 2 c.c. of 1·11 per cent. solution of calcium chloride. The usual calcium effects ensued, see Fig. 2. B. Then to the 200 c.c. of circulating fluid I added 2 c.c. of 1·685 per cent. solution of cæsium chloride. At first the fusion increased much. Five minutes after the addition of cæsium I obtained the trace shown at C.

The contractions then grew gradually more regular and about twelve minutes after adding the cæsium the beats became just like those with blood mixture. An hour after the replacement the ventricle beat spontaneously and its beats were equally good and of the same character as with blood mixture: see D. Here the cæsium before it had time to expend its full antagonistic effect, caused much irregularity in the character of the beat, but when the antagonism became complete, the contractions assumed a natural character just like those with blood mixture.

Cæsium, then, in spite of its close chemical affinity to potassium, differs considerably from potassium. Cæsium, indeed, produces for the most part the opposite effects to potassium, broadening the trace, rounding its top, inducing fusion; yet in spite of this great difference in its action still cæsium, like potassium, possesses the property of obviating the effect of calcium on the dilatation.

Next I endeavoured to ascertain whether with strontium and cæsium together we could constitute a fluid able to sustain the heart's contractility.

I replaced blood mixture by 100 c.c. saline solution containing 4 c.c. of 1 per cent. solution of sodium bicarbonate, 1 c.c. of 1·685 per cent. solution of cæsium chloride and 1 c.c. of 1·586 per cent. solution of strontium chloride. The contractions grew much broader and in about ten minutes, the dilatation became much prolonged and the contractions grew feeble. I then added 1 c.c. of 1·11 per cent. solution of calcium chloride and the contractions at once improved and the trace soon quite recovered its original height, and the delay in dilatation disappeared, the trace remaining only a little broader than with blood mixture.

We find then that strontium and cæsium cannot sustain the heart's contractility, and that on the addition of equivalent molecular quantity of lime the strontium effects are removed, the contractions recover and become normal again.

As cæsium is so similar to barium in its action on the ventricle, I resolved to ascertain if barium could take the place of cæsium and when associated with a lime salt could sustain the contractility of the ventricle. I replaced blood mixture by 100 c.c. of saline solution containing 2 c.c. of 1 per cent. solution of sodium bicarbonate, 1 c.c. of 2·08 per cent. solution of barium chloride and 1 c.c. of 1·11 per cent. solution of calcium chloride. The beats became broader and fused and grew much weaker. I then added 1 c.c. of 1·685 per cent. solution of cæsium chloride. The fusion ceased, and at first lime effects persisted, for the dilatation continued much delayed, but this condition soon disappeared and the trace became almost the same as with blood mixture.

Here, then, as might be expected, in spite of the close similarity in their action on the ventricle, barium cannot replace cæsium, see Fig. 3.

Cæsium differs in part from both potassium and rubidium in respect to its influence on the effect from continuous faradization. Under the influence of cæsium chloride, faradization does not inhibit the cardiac contractility. I made experiments exactly similar to those with rubidium, using a 1·685 per cent. solution of cæsium chloride. Additions from time to time of this solution produce gradual loss of excitability of the cardiac tissue for the faradic stimulus. Starting with a good tetanus, this gradually lessened and at last the faradization is without effect, the existing rhythm being unmodified. This occurred after the addition of 3 c.c. of the cæsium chloride solution, but the inhibitory effect was not seen even after 4 c.c. though the ventricle was practically arrested in full diastole; it was possible to see for each period of faradization, a faint corresponding waviness of the trace, indicating faint contractions.

Dr Sainsbury tested the effect of cæsium on the period of diminished excitability by experiments performed exactly like those with rubidium.

The period of latency was somewhat increased but the effect was not very marked. The period of diminished excitability was very decidedly increased, the metronome being advanced from 15, which position was effective up to the top of the scale (0) and yet the second stimulus remained without effect. This occurred after the addition of 2 c.c. of the cæsium solution. In this experiment the ligature was placed well below the auriculo-ventricular groove, through the substance of the ventricle, yet in spite of this spontaneous contractions set in after the drug was commenced and this increase in the period of diminished excitability occurred in spite of the increased frequency of the spontaneous contractions.

Cæsium chloride, then, in its physiological action on the ventricle, only corresponds to potassium chloride in these respects, both obviate the delay in dilatation caused by calcium salts and both increase the period of diminished excitability and so prevent tetanus from continuous faradization.

It is interesting to compare the effect of chemical similarity on physiological similarity in the group calcium, strontium and barium and in the group including potassium, rubidium and cæsium.

Strontium is chemically more allied to calcium than barium is to calcium and whilst strontium is in its physiological action very similar to calcium, barium is far less similar to calcium. So it is with the group potassium, rubidium and cæsium. Rubidium is chemically more allied than cæsium to potassium and yet rubidium is almost identical in its physiological action with potassium, whilst cæsium differs in most of its physiological effects from potassium.

Plate XXII.

FIG. 1.

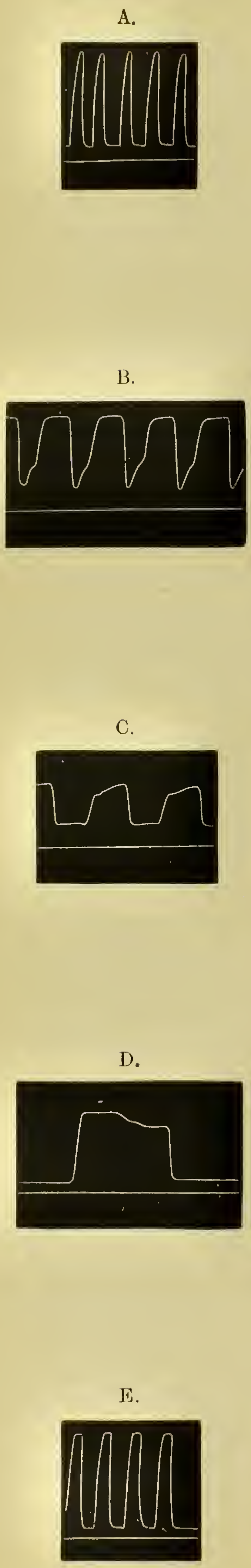


FIG. 2.

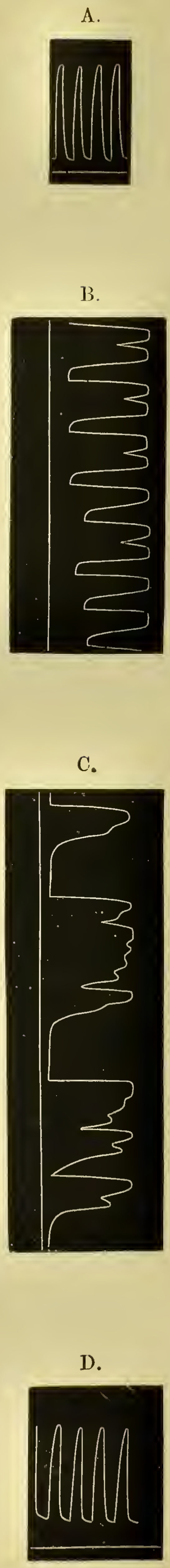
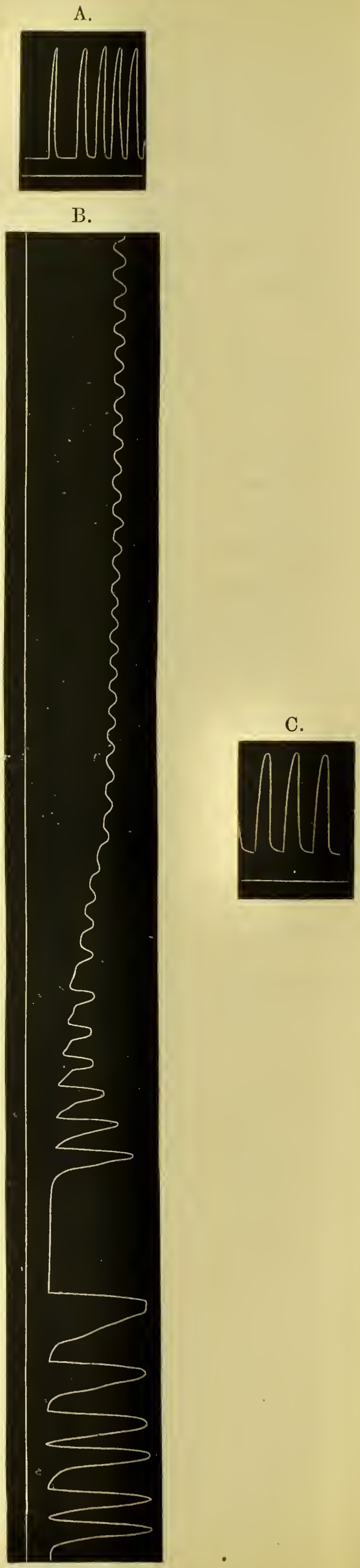


FIG. 3.



EXPLANATION OF FIGURES. PLATE XXII.

Fig. 1.

A. Trace with blood mixture.

B. Trace taken ten minutes after the substitution of blood mixture by 200 c.c. of saline solution containing 2 c.c. of 1·685 per cent. of cæsium chloride.

C. Ten minutes after the addition of 3 c.c. of a 1 per cent. solution of sodium bicarbonate to 150 of the above.

D. After the addition of 1 c.c. of a 1 per cent. solution of potassium chloride to 150 of circulating fluid and subsequently of 1·5 c.c. of a 1·586 per cent. solution of strontium chloride.

E. After the addition of 1·5 c.c. of a 1·11 per cent. solution of calcium chloride.

Fig. 2.

A. Trace with blood mixture.

B. Trace about fifteen minutes after replacing blood mixture with 200 c.c. saline solution containing 4 c.c. 1 per cent. solution of sodium bicarbonate and 2 c.c. 1·11 per cent. solution of calcium chloride.

C. Trace five minutes after adding 2 c.c. of 1·685 per cent. solution of cæsium chloride to 200 of circulating fluid.

D. Forty-five minutes after the addition of cæsium chloride and an hour after the replacement of blood mixture by saline solution containing sodium bicarbonate and calcium chloride.

Fig. 3.

A. Trace with blood mixture.

B. Effect of replacing blood mixture by saline containing chloride of calcium, chloride of barium and sodium bicarbonate.

It shews also the effect of adding cæsium to the circulating fluid; the cæsium chloride was added at the point indicated by an arrow.

C. Trace twenty minutes after adding cæsium chloride and forty minutes after replacing blood mixture by saline containing barium chloride and calcium chloride.

[*From the Journal of Physiology. Vol. V. No. 2.*]

CONCERNING THE INFLUENCE OF SALINE MEDIA ON FISH, ETC.

By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.*

THIS paper[1] is a record of experiments showing the influence of the saline constituents of fresh water on fishes.

Whilst fish will live for weeks unfed in tap water, they die when placed in distilled water in a few hours, minnows for instance on an average of 4·5 hours. The addition however either of a sodium or a calcium salt in minute proportions greatly prolongs their life. Sodium chloride, sodium bicarbonate and potassium chloride added singly to distilled water sustain life for about the same time, namely from one to two days. Sodium bicarbonate prolonging life rather longer than either of the two other salts.

Calcium chloride added to distilled water sustains life much longer than either corresponding quantities of sodium or potassium salts.

The addition of sodium bicarbonate greatly enhances the life-prolonging effect of calcium chloride. This combination indeed appears as capable of sustaining life as tap water.

I find that whilst a reduction of the relative quantity of salts below a certain point renders water incapable of sustaining life, that on the other hand fish, as minnows and sticklebacks, can exist in fluid containing a considerable excess of salts over that common in river waters. I find too that by gradually reducing the quantity of sodium salts I can habituate minnows and sticklebacks to live in a fluid containing only a calcium salt or calcium chloride. Sticklebacks which are anadromous become, as one would naturally expect, much more readily habituated to a fluid of a different composition to that to which they are accustomed and far more readily than minnows.

Many fish are endowed with the capacity to live in waters of very variable composition—in waters which speedily kill other fish.

Day[2] says that whilst Pleuronectidiæ (Flat-fishes) are mostly marine and found in shallow water with a sandy bottom; some, as the common flounder, are anadromous, that is ascend rivers and can live in fresh water. He states[1] that the diamond plaice thrives in fresh water ponds.

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[1] A short notice of these experiments appeared in the *Journal of Physiology*, 1883.

[2] *Fishes of Great Britain and Ireland*, Part v. 1882, p. 5.

Soles too, he says, ascend rivers above the tide-way and remain and flourish there throughout the year.

Mr Harvie Brown caught at Durness several so-called sea trout from a sea pool, its water being quite fresh at low tide and salt or brackish at high tides.

Mullet in hot weather ascend the Avon for twenty miles, far beyond the influence of even a spring tide[2].

Stickleback and codfish are anadromous.

In a subsequent paper I hope to show that the composition of the water greatly influences the growth and development of the ova and larvæ of frogs, and therefore probably of fishes too. With ova and larvæ of frogs I can not only arrest growth but development in spite of growth, and can produce various distorted forms, although these after a month or more generally die and I have not yet succeeded in rearing frogs from them. Yet it seems not unlikely that slighter variations occurring through some temporary alteration in the composition of the water of streams may be able to live and so lead to the formation of species.

The same holds probably with fishes, for the composition of the water influences the hatching of salmon ova, for they[3] will not hatch in the sea though sometimes they are deposited there.

Saline water of sp. gr. 1016 is fatal to impregnated ova and to young fish, and when the water is diluted to sp. gr. 1007 the advanced ova may be hatched in it and young fish kept alive for a few days, though with diminution of powers and vitality. Day also states that breeding in confined places is believed to cause a malformation of the gill covers, and a similar malformation I can induce in frog larvæ by certain conditions to be described subsequently.

The composition of water modifies the nutrition and growth of well developed fish. Thus the flounder[4] found in rivers above tidal influences has soft flesh, due to its more rapid growth.

On ascending a river to breed the flesh of salmon deteriorates, becoming softer and paler, and the silvery lustre of the scales changes to a muddy hue. Some streams invariably yield trout with red flesh, other streams yield trout with white flesh.

When the anadromous forms of salmon descend to the sea, they lose the transverse bars and become silvery.

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[1] *Ibid.* p. 27. [2] Day, p. 229, Vol. III. [3] Day, *ibid.* [4] Day, *ibid.* p. 35.

Again, in some salmon (Salmo cambricus) if retained some time in fresh water ponds, the pectoral fins become more pointed than in others which are allowed to go to the sea.

In California the Salmo tridens inhabits all the brooks and rivers; it descends every autumn to the sea, where it loses its spots and changes its colour to a steel blue. Day further says the colour, depth, and character of the water have an influence on the Salmonidæ. Some caught in dark holes or caves were nearly black.

Mr J. Harvie Brown[1] observes that at a far inland locality in Sutherlandshire, brown trout, dark and spotted, were caught in 1877, and introduced into a chain of lochs in the same country, which have their sources in innumerable springs of clear water from granite and limestone mountains, chiefly the former. The fish became in a single year silvery, covered with minute bright scales like sea trout, and grew to the size of one pound weight in twelve months. The food in the loch is shellfish and tadpoles, and the bottom granite, gravel and sand.

Mr Brown also observed that continuous pollution of water through the agency of paper mills causes a contraction of the rays of the tail fins.

Lord Home observes of the two streams, the Whiteadder and its tributary the Blackadder, that the former flows along a very rocky and gravelly bed; while the latter rises in mosses and also traverses them in the first half of its course, but subsequently along a rich and highly cultivated district. The trout in the Whiteadder are silvery, while those from the Blackadder are dark with orange fins.

In loch Islay there exists a race of tailless trout; this peculiarity has been traced to the action of deleterious matter in some streams, but its nature is not stated.

The gold fish is remarkable for many varieties of colour subject to the influence of temperature, water, &c. A correspondent in *Land and Water* remarks that for the purpose of inducing colour the chief materials needed are iron, gall-nuts and tan; and by proper treatment a fish, originally white and red, can be rendered black, white and red.

The growth of the smelt is said to be frequently much retarded when passing from marine to a fresh water state of existence.

Moss and peat[2] and a muddy bottom cause salmon to assume a dark tint; clear water in rapid rivers and lakes and a pebbly bottom give the fish a silvery colour.

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[1] Day, *loc. cit.* p. 100, Part VI.

[2] *Land and Water*, 1882, p. 147.

High altitudes[1] and a weedy sandy bottom appear to retard the development of trout.

These changes in many instances are not due to habituation, for some fish will pass at once from one medium to another of a very different composition, as many of the previous instances illustrate. On the other hand some fish require a gradual change to become habituated to a different medium. For instance[2] the double spotted Goby lives for a long time in fresh water when gradually accustomed to it; but when suddenly placed in cold fresh water it becomes apparently asphyxiated. In this instance the new medium appears to affect the gills as distilled water affects the gills of fresh-water fishes. The Gobius niger is capable of living in fresh water or salt water even if suddenly changed from one to the other[3].

The composition of the water very probably has much bearing on salmon disease; for Day asserts that a fungus which attacks salmon in fresh water seems to get cured by going down to the sea, and sea water generally appears to check the disease.

In the experiments recorded in this paper observations were taken at about 9 a.m. at noon and between 6 and 8 p.m.

Except where the contrary is stated, the fish were kept in the same testing water throughout the experiment; and except where the contrary is stated, I always placed the same number of fish in the same quantity of fluid, for instance six minnows or twelve sticklebacks or three gold fish to the 1000 c.c.

During their probation, the fish were unfed.

The solutions of calcium chloride, of sodium bicarbonate, of potassium chloride, each contained respectively 1 p.c. of salt.

Minnows, sticklebacks and gold fish, eels, &c. cannot live in distilled water.

In various experiments I tested the effect of distilled water on 22 sticklebacks and 28 minnows.

The sticklebacks died in an average time of 14 hours.

The minnows died in an average time of 4·5 hours.

This inability to support life may be due to a too small quantity of oxygen or the absence of inorganic salts in the distilled water.

It is not due to inadequate amount of oxygen, for before and during the experiments the distilled water was freely exposed to the air; and further, on adding inorganic salts to the distilled water, its power of sustaining life is increased; moreover the addition of certain salts in proper proportions to distilled water renders it capable of sustaining life in the same degree as pipe water, in which minnows and sticklebacks and gold fish will exist for weeks without food.

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[1] *Ibid.* 1882, Dec. 2. [2] Day, Vol. III. p. 147. [3] *Ibid.* p. 165.

The inability of distilled water therefore to sustain fish life is due to the absence of certain inorganic salts.

As one would expect, these salts must be present in a certain per centage proportion; at least there is a minimum per centage below which the water is incapable of sustaining fish life for any length of time, though fish will live in water containing a considerably larger quantity of these salts. I find that minnows placed in a fluid consisting of one part of tap water to two parts of distilled water die on an average in 43 hours; the salts greatly diluted sustained life longer than distilled water, but could not sustain life for any length of time.

I often tested the distilled water and got others skilled in this matter to test it, and we thought the distilled water gave a very faint acid reaction. I made a series of experiments to learn if the death of the fish in distilled water is due to the faint acidity. I placed 1000 c.c. of tap water in three vessels; into the first I dropped 6, into the second 12, into the third 20 drops of 10 p.c. solution of acetic acid. Each of these solutions gave decided acid reaction, far greater than the distilled water. Twenty-four hours afterwards, the three minnows placed in each vessel were quite natural.

In five control experiments to test how long minnows will live in tap water I obtained the following results.

I. Of 11 minnows all were alive on the seventh day.

II. Of 5 minnows all were alive on the ninth day.

III. Of 12 minnows one died on the 13th day and 11 were alive on the 15th day.

I now give the results of many testings of the effect of common salt, of sodium bicarbonate, of potassium chloride, of calcium chloride, and of various combinations of these salts, in sustaining life of minnows.

Experiments with sodium chloride.

Six minnows in a solution consisting of 10 c.c. saline solution (0·75 p.c.) in 1000 c.c. distilled water died on an average in 11 hours.

Six fish in a solution consisting of 20 c.c. of saline solution in 1000 c.c. of distilled water died on an average in 14 hours.

Six fish in a solution consisting of 30 c.c. of saline solution to 1000 c.c. of distilled water died on an average in 29 hours.

Eighteen fish in a solution consisting of 40 c.c. of saline solution to 1000 c.c. of distilled water died on an average in 19 hours.

Six fish in a solution consisting of 50 c.c. of saline solution to 1000 c.c. of distilled water died on an average in 28 hours.

Six fish in a solution consisting of 60 c.c. of saline solution to 1000 c.c. of distilled water died on an average in 30 hours.

Experiments with sodium bicarbonate.

Twenty-six fish in a solution consisting of 20 c.c. of sodium bicarbonate solution to 1000 c.c. of distilled water died on an average in 16 hours, one living for three days and a half.

Six fish in a solution consisting of 30 c.c. of sodium bicarbonate solution to 1000 c.c. of distilled water died on an average in 50 hours.

One died on the 4th day,
One „ „ 6th day.

Five fish in a solution consisting of 40 c.c. of sodium bicarbonate solution to 1000 c.c. of distilled water died on an average in 47 hours.

Six fish in a solution consisting of 50 c.c. of sodium bicarbonate solution to 1000 c.c. of distilled water died on an average in 31 hours.

Experiments with potassium chloride.

Twelve fish in a solution consisting of 10 c.c. of potassium chloride solution to 1000 c.c. of distilled water died on an average in 12 hours.

Six fish in a solution consisting of 20 c.c. of potassium chloride solution to 1000 c.c. of distilled water died on an average in 17 hours.

Twelve fish in a similar solution.

Nine died on an average of 30 hours,
Two on the 8th day, and
One on the 9th day.

Twelve fish in a solution consisting of 30 c.c. of potassium chloride solution died on an average in 17 hours.

Twelve fish in a solution consisting of 50 c.c. of potassium chloride solution to 1000 c.c. of distilled water died on an average in 12 hours.

Twelve fish in a solution consisting of 60 c.c. of potassium chloride solution to 1000 c.c. of distilled water died on an average in 32 hours.

Experiments with chloride of calcium.

Forty-one fish in a solution consisting of 10 c.c. of calcium chloride solution to 1000 c.c. of distilled water died on an average in 24 hours

Twelve fish in a solution consisting of 20 c.c. of calcium solution to 1000 c.c. of distilled water died on an average in 26 hours.

Fifteen fish in a solution consisting of 30 c.c. of calcium chloride in a 1000 c.c. of distilled water, six died on an average of 47 hours.

Nine were alive on the 12th day.

Six fish in a solution consisting of 40 c.c. of calcium chloride solution to the 1000 c.c. of distilled water died on an average of 60 hours.

One died on the 5th day,
One „ „ 6th day.

Ten fish in a solution consisting of 50 c.c. of calcium chloride solution to the 1000 c.c. of distilled water, seven died on an average of 33 hours.

Three were alive on the 9th day.

Eleven fish in a solution consisting of 60 c.c. of calcium chloride solution to the 1000 c.c. distilled water, nine died on an average of 90 hours.

Two were alive on the 9th day.

Experiments with calcium chloride and potassium chloride.

Twenty fish in a solution consisting of 10 c.c. of calcium chloride solution and 5 c.c. of potassium chloride solution to the 1000 c.c. of distilled water died on an average

Nineteen in 23 hours,
One was alive on the 6th day.

Twelve fish placed in a solution consisting of 10 c.c. of calcium chloride and 20 c.c. of potassium chloride solution to the 1000 c.c. of distilled water died on an average in 61 hours.

Experiments with calcium chloride and sodium bicarbonate.

Twenty-one fish in a solution consisting of 10 c.c. of calcium chloride solution and 20 c.c. of sodium bicarbonate solution to the 1000 c.c. of distilled water.

Sixteen died on an average of 47 hours,
One alive on the 5th day,
Four alive on the 9th day.

Ten fish in a solution consisting of 20 c.c. of calcium chloride solution and 20 c.c. of sodium bicarbonate solution to the 1000 c.c. distilled water.

One died on the 3rd day,
Two „ „ 17th day,
Seven alive on the 19th day.

Experiments with calcium chloride, sodium bicarbonate, and potassium chloride.

Eleven fish in a solution consisting of 10 c.c. of calcium chloride solution, 10 c.c. of potassium chloride solution, and 20 c.c. of sodium bicarbonate solution to the 1000 c.c. of distilled water.

Four died on an average in 26 hours,
Seven were alive on the 9th day.

Nineteen fish in a solution consisting of 20 c.c. of calcium chloride solution, 20 c.c. of sodium bicarbonate solution, and 10 c.c. of potassium chloride solution to the 1000 c.c. of distilled water.

Three died on an average in 36 hours.
Four were alive on the 7th day,
Twelve ,, ,, 12th day.

Twelve fish in a similar solution.

All alive on the 21st day.

It is evident that the addition to the distilled water of any one salt prolongs fish life. The effect of sodium chloride, of sodium bicarbonate, of potassium chloride is much the same, whilst calcium chloride sustains life for a much longer term. The first-named salts sustain life from one to two days, whilst a corresponding dose of calcium chloride sustains life for many days; for instance with 30 c.c. of 1 p.c. solution added to 1000 c.c. of distilled water six fish died on an average of 47 hours, whilst on the 12th day nine were still alive.

The addition of sodium bicarbonate to calcium chloride greatly enhances the life-sustaining effects of this salt. For instance 10 c.c. of 1 p.c. solution of calcium chloride per 1000 c.c. of distilled water sustains life on an average for 24 hours, but the addition of 20 c.c. of 1 p.c. solution of sodium bicarbonate gave these notable results; 16 fish died on an average of 47 hours, one was alive on the fifth day and four were alive on the ninth day.

Again, with 20 c.c. of sodium bicarbonate solution and 20 c.c. of calcium chloride solution, of ten fish one died on the third day, two on the seventeenth, and seven were alive on the nineteenth day.

The addition of potassium chloride to calcium chloride and sodium bicarbonate sustains life in a still more marked degree, though the influence of the potassium chloride is only slight.

In three experiments with a solution consisting of 20 c.c. of calcium chloride solution, 20 c.c. of sodium bicarbonate solution, and 10 c.c. of potassium chloride solution to the 1000 c.c. of distilled water,

I. One fish died in 11 hours,
Ten „ „ 48 hours,
Four were alive on the 7th day.

II. Twelve fish were all alive on the 12th day.

III. Twelve fish were all alive on the 21st day.

In these experiments I often noticed that one, two, or more of the minnows greatly outlived the rest.

For instance, in an experiment with six minnows placed in 1000 c.c. distilled water containing 30 c.c. of 1 p.c. solution of sodium bicarbonate, two died in 11 hours, one in 20 hours, one in 33 hours, one in 90 hours, one in 146 hours.

Again, in an experiment with fifteen fish placed in solution containing 30 c.c. of 1 p.c. solution of calcium chloride to the 1000 c.c. distilled water, six died on an average of 47 hours, whilst nine were alive and active on the twelfth day.

This prolongation of life in some of the fish much beyond their fellows must be due either to habituation or because the dying fish give up some constituent, probably inorganic salts, to the fluid in sufficient quantity to sustain the life of the survivors.

I put 24 minnows in 1000 c.c. of distilled water, that is four times more fish than in the previous experiments. Most died in the course of six or seven hours, nine were living at the end of 24 hours, five died in 48 hours, and two were alive and well at the expiration of 72 hours. These two survivors I placed in a fresh supply of distilled water and they were both dead in six hours.

It is obvious here that the prolonged survival of two of the minnows was not due to habituation to the distilled water, for they died speedily when placed in a fresh supply of it. It is probable that the other fish gave up something to the water, probably inorganic salts, which enabled the water to sustain life; and that this is the correct view is, I think, exemplified in this continuation of the experiment. Into the 1000 c.c. of fluid which previously contained the 24 minnows, I put six fresh minnows, and all were alive and active seven days afterwards. It is obvious then that the first fish gave up something to the water to increase its life-sustaining powers.

I repeated the preceding experiment with a slight modification.

I placed 24 minnows in 1000 c.c. distilled water.

Thirteen died in 4 hours.
One ,, ,, 10 ,,
Three ,, ,, 16 ,,
One ,, ,, 30 ,,
One ,, on the 4th day.
One ,, ,, 5th ,,

Three were alive and active on the 8th day.

I tested the water which had contained the fish for lime by adding a little oxalate of ammonium solution, and produced a slight opalescence, indicating the presence of a minute trace of lime though decidedly less lime than tap water contains diluted with two parts of distilled water. I then placed the three surviving fish in one part of tap water and two parts of distilled water; one died in 14 hours, one in 28 hours, and one in 34 hours. Therefore I conclude that the prolonged life of some of the fish in distilled water is not due simply to the abstraction of a lime salt but also of other inorganic salts or possibly to organic matter.

In an experiment with six minnows placed in 1000 c.c. distilled water containing 40 c.c. of saline solution (0·75 p.c. common salt), five died on the second day, but one lived on to the sixth day and was then put into a fresh solution of the same composition and died in less than 24 hours. This extension of life in one fish cannot be attributed to habituation.

I placed twelve minnows in 1000 c.c. of distilled water with 30 c.c. of sodium bicarbonate solution; another twelve fish in 2000 c.c. of distilled water with 60 c.c. of sodium bicarbonate solution, and yet another twelve fish in a 1000 c.c. of distilled water with 30 c.c. of sodium bicarbonate solution.

The first and second lot of fish I allowed to remain in the same fluid throughout the experiment, but the second lot had twice as much fluid as the first, and consequently the salts, &c. separated from its complement of fish would be more diluted than with the first batch and the fish should die sooner.

With the third lot I replaced the fluid by a similar quantity of the same solution twice a day.

The twelve minnows placed in 1000 c.c. of solution lived on an average 65 hours.

The twelve placed in double the quantity of fluid (2000 c.c.) died on an average in 23 hours.

The twelve whose solution was changed twice a day died on an average in 15 hours.

Into the 2000 c.c. of fluid in which twelve minnows had died, I then placed another 12 minnows.

One died in 34 hours.
,, ,, 55 ,,
One died on the 4th day.
Two ,, ,, 5th ,,
,, ,, ,, 6th ,,
One ,, ,, 7th ,,
Four alive on 9th day.
Three ,, 12th day.

I then placed the three surviving fish in 1000 c.c. distilled water and 30 c.c. of sodium bicarbonate solution. Two were dead in 15 hours and the last died in 27 hours, showing that their prolonged life was not due to habituation to the fluid, but that the former tenants had given up some substances to the fluid and rendered it capable of supporting life.

It is obvious that in these experiments the fish gave to the water some substances capable of supporting the life of other fishes. As fish cannot live without lime and potash and soda salts, it is evident at least that the deceased fish gave enough of these salts to the water to enable it to sustain the life of their successors.

Yet the quantity of lime thus yielded cannot be large, for when 24 minnows are placed in distilled water whilst most of them die yet some live many days. Now this fluid when filtered and treated with oxalate of ammonia becomes just perceptibly opalescent, far less so than tap water itself and even less than tap water diluted with two parts of distilled water, and this mixture will not, I have shown, sustain fish life beyond an average of 43 hours.

I next record a few experiments made with sticklebacks during the autumnal and winter months of 1883.

Twenty-two sticklebacks placed in distilled water died on the average in 14 hours.

The shortest interval was six, the longest twenty-eight hours.

With distilled water containing for each 100 c.c. 1 c.c. of calcium chloride solution,

Twenty-one fish, ten died on the average in 59 hours.
Two were alive on the 3rd day,
Nine ,, ,, 9th ,,

With distilled water containing for each 100 c.c. 1 c.c. of calcium chloride solution and 1 c.c. of potassium chloride solution,

Of twenty fish

Thirteen died on the average of 63 hours.
One was alive on the 3rd day.
,, ,, ,, 7th ,,
Five ,, ,, 9th ,,

on which day I discontinued the experiment.

With distilled water containing 2 c.c. of calcium chloride solution per 100 c.c.,

Three died in 19 hours.
Two died on the 3rd day.
One ,, ,, 4th ,,
Two ,, ,, 5th ,,
One ,, ,, 8th ,,
,, ,, ,, 16th ,,
,, alive ,, 21st ,,

This surviving fish was placed in a fresh solution of the same composition and was alive four days after.

With distilled water containing for each 100 c.c. 3 c.c. of calcium chloride solution,

One died in 13 hours.
,, ,, 54 ,,
Four died on 3rd day.
One ,, 5th ,,
,, ,, 14th ,,
Three alive on 21st day.

The three surviving fish were placed in fresh fluid of the same composition.

One died on the 3rd day.
Two were alive on 4th day.

With distilled water containing for each 100 c.c. 4 c.c. of calcium chloride solution,

Four died on an average in 14 hours.
One ,, ,, ,, on 3rd day.
,, ,, ,, ,, ,, 10th ,,
,, ,, ,, ,, ,, 21st ,,
Five were alive on 21st day.

The surviving fish were placed in a fresh quantity of fluid of the same composition, and were all alive four days after.

With twenty sticklebacks placed in tap water, all lived. With two I concluded the experiment on the 3rd day, with three on the 7th, and with fifteen on the 9th day.

In another experiment with eleven fish,

Three died on the 6th day.
One ,, ,, 11th ,,
,, ,, ,, 21st ,,
Nine were alive on 25th day.

Sticklebacks therefore live in distilled water about four times longer than minnows.

Lime chloride, then, prolongs the life of sticklebacks much more markedly than of minnows, and a smaller quantity has notably greater influence on sticklebacks than on minnows.

With 1 c.c. of calcium chloride to 100 c.c. of distilled water minnows die on an average in 24 hours.

With 2 c.c. of calcium chloride to 100 c.c. of distilled water minnows died on an average in 26 hours.

With 3 c.c. of calcium chloride solution to 100 c.c. of distilled water, of 15 fish

Six died on an average in 47 hours.
Nine were alive on the 12th day.

Gold Fish.

Three gold fish in 1000 c.c. of distilled water.

One died in 46 hours.
Two ,, 54 ,,

The fluid renewed twice, once each day.

Their weights respectively were 7, 7 and 4 grammes.

On the first day, as with other fish, the fluid became very flocculent from the presence of epithelium and mucous cells.

Three fish in 1000 c.c. of distilled water containing 30 c.c. of calcium chloride solution.

One died on the 9th day.
,, ,, ,, 10th ,,
,, ,, ,, 16th ,,

The fluid renewed eight times.

Three fish in 1000 c.c. of distilled water containing 20 c.c. of calcium chloride and 20 c.c. of sodium bicarbonate solutions.

One died on the 13th day.
Two alive on the 23rd day.

Fluid renewed eight times.

Three fish placed in 1000 c.c. of tap water.

One died on the 21st day.
Two alive on the 23rd day.

Fluid renewed eight times.

In these trials I renewed the fluid frequently, for experiments with minnows show that they give up some substance to the water rendering it more capable of sustaining life and heat.

This is the case likewise with gold fish, as shown in these experiments, in which the fluid was not renewed throughout the operation.

Of three fish in 800 c.c. distilled water,

One died in 4 days.
" " 6 "
" alive on 15th day

Of three fish in 800 c.c. distilled water containing 8 c.c. of calcium chloride solution,

One died in an hour.
" " 28 hours.
" " on 4th day.

Of three fish placed in 800 c.c. distilled water containing 8 c.c. of calcium chloride and 8 c.c. of potassium chloride solutions,

One died on the 4th day.
" " 5th "
" " 6th "

Of three fish placed in 800 c.c. of tap water,

One died in 24 hours.
" " on the 5th day.
" " " 8th "

Like minnows and sticklebacks, gold fish cannot live in distilled water, but the addition of small quantities of salts greatly increases their duration of life. In fact the addition of 1 c.c. of calcium chloride and 1 c.c of sodium bicarbonate to the 100 c.c. distilled water sustains life as well as tap water.

Eels.

Feb. 4. An eel weighing 115 grammes in 5000 c.c. distilled water died on 3rd day.

Feb. 4. An eel weighing 250 grammes in 5000 c.c. distilled water died 2nd day.

Feb. 5. An eel weighing 305 grammes in 5000 c.c. distilled water died 2nd day.

The fluid became, as with other fish, very flocculent.

In the foregoing experiments the water was changed daily.

Feb. 2. An eel placed in tap water which was renewed daily died on 10th day.

It appears then that distilled water kills eels like other fish very speedily.

I placed a dace weighing 3 oz. in 9000 c.c. distilled water, and next morning found the fish dead and the water very flocculent. Another dace in tap water seemed quite lively after twenty-four hours' immersion.

Newts.

Feb. 7. Four newts placed in 2000 c.c. tap water, renewed daily.
One died on the 10th day.
Three alive on the 13th day.

Feb. 7. Five newts in 2000 c.c. distilled water, the water changed daily.
Two died on 3rd day.
,, ,, 4th ,,
One ,, 5th ,,

Feb. 7. Seven newts in 2000 c.c. distilled water, renewed daily.
Three died on 2nd day.
One ,, 4th ,,
Two ,, 5th ,,
One ,, 6th ,,

Feb. 7. Three newts in 2000 c.c. distilled water, renewed daily.
One died on 4th day.
Two ,, 5th ,,

Distilled water therefore, though in a less degree, is fatal to newts.

We have then ascertained that fish placed in artificial waters live a very variable period, some die in a few hours, others survive several days. This prolonged life in some instances, I have shown to be due to the fish giving up some organic or inorganic substances to the waters which render them more capable of sustaining life.

The possibility however that fish may become habituated to the fresh media has led me to conduct a large series of experiments to endeavour to settle the point whether fish can adapt themselves to strange media with a composition different from the waters which constitute their natural habitats.

The greater number of experiments I made with minnows, with however but little success. It is not necessary here to record the failures, but I will describe my solitary successful case.

Oct. 14. I placed 24 minnows in 4000 c.c. distilled water containing 40 c.c. of calcium chloride and 20 c.c. of potassium chloride solutions.

On the 6th day only one survived, and on this day I renewed the fluid and again renewed it on the 11th day.

On the 13th day I began to reduce the potassium chloride, and placed the minnow in 1000 c.c. distilled water containing 10 c.c. calcium chloride solution and 4 c.c. potassium chloride solution.

On the 14th day I changed the fluid, reducing the potassium chloride to 3 c.c. per 1000. On the 16th I reduced it to 2 and on the 18th to 1 c.c., and on the 19th day I omitted the potassium chloride, so that the fluid consisted of 1000 c.c. distilled water with 10 c.c. calcium chloride 1 p.c. solution.

On the 24th day I renewed the fluid and on the 34th day the minnow was alive and active, so that it had lived unfed for 35 days in an artificial medium, and for 15 days this medium consisted simply of distilled water and calcium chloride in the proportion of one part of calcium chloride to 1000 parts of water.

In the many unsuccessful experiments no doubt I lowered the quantity of salts too rapidly.

I have now to refer to some experiments made with sticklebacks recorded in this paper.

I placed minnows in different solutions of calcium chloride in distilled water, keeping them in the unchanged fluid.

In three experiments, on the 21st day nine fish survived.

I then renewed the solutions, and four days afterwards eight were alive.

Sticklebacks are considered hardy fish, whilst minnows are spoken of as delicate fish and kept alive with difficulty. These experiments partially explain this difference in hardiness, for sticklebacks will live in solutions which prove quickly fatal to minnows.

I next tried the effect of adding calcium chloride to tap water.

I placed 12 minnows in 2000 c.c. tap water, to which I added 20 c.c. calcium chloride 1 p.c. solution.

They were all alive on the 9th day.

I placed 13 minnows in 2000 c.c. tap water containing 200 c.c. of 1 p.c. solution of calcium chloride, and all were alive on the 12th day.

Placed 12 minnows in 2000 c.c. tap water containing 40 c.c. of 5 p.c. solution of calcium chloride.

Two died on the 1st day.
One ,, ,, 5th ,,
,, ,, ,, 11th ,,
,, ,, ,, 12th ,,
Seven alive on the 12th day.

Placed 12 minnows in 2000 c.c. tap water containing 50 c.c. 5 p.c. solution of calcium chloride.

Four died on the 1st day.
Eight were alive on the 14th day.

Placed 12 minnows in 2000 c.c. tap water containing 60 c.c. 5 p.c. solution of calcium chloride.

One died on the 4th day.
Eleven alive on the 12th day.

Placed 11 minnows in 2000 c.c. tap water containing 100 c.c. of 5 p.c. solution of calcium chloride.

All alive on the the 11th day.

Placed 12 minnows in 2000 c.c. tap water containing 150 c.c. of 5 p.c. solution of calcium chloride.

All were alive on the 9th day.

Placed 12 minnows in 2000 c.c. tap water containing 200 c.c. of 5 p.c. solution of calcium chloride.

One died on the 2nd day.
" " " 3rd "
Ten alive on the 14th day.

Placed 12 minnows in 2000 c.c. tap water containing 250 c.c. of 10 p.c. calcium chloride solution.

All dead by the 9th day.

Placed 12 minnows in 2000 c.c. tap water containing 300 c.c. of 10 p.c. solution of calcium chloride.

All were dead in 24 hours.

We see then that to sustain the life of fish, the water in which they live must contain a minute quantity of various salts, for instance one in 5,000 to 10,000 of calcium and sodium salts. That in water containing a smaller quantity fish languish and die, and they die still sooner in distilled water. On the other hand they can live in a great excess of lime over the minimum essential to life.

How does distilled water destroy fish life? Probably in one of two ways or in both.

Lime is essential to muscular contraction[1] and probably, I would suggest, to the performance of any function of the body; potash and soda also are probably equally essential. It may be that these salts are withdrawn from the blood and tissues through the surface of the body, but especially through the gills. On the other hand it may be that distilled water destroys by acting osmotically on the tissues, and especially on the tissues of the gills, mainly by interference with respiration.

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[1] *Journal of Physiology*, Vol. IV.

In favour of the first view I may draw attention to the experiments showing that fish give up some material to the distilled water, enabling it to sustain life, and that this material consists probably of sodium, calcium, and possibly of potassium salts.

The second view is supported by the fact that fishes, soon after immersion in distilled water breathe laboriously, the water soon becomes opaque with flocculi; and those solutions best capable of sustaining life become least flocculent.

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ON THE MUTUAL ANTAGONISM BETWEEN LIME AND POTASH SALTS, IN TOXIC DOSES.

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In previous communications[1] I showed that in physiological quantities there is a mutual antagonism between lime and potash salts, and that through this very antagonism the proper contraction of the heart is maintained. When lime is unantagonized by a potash salt, then great delay of the cardiac dilatation ensues, so that before the completion of dilatation another contraction begins. The beats thus become fused and the ventricle not dilating completely, or even very slightly, the amount of blood propelled with each systole is much reduced in quantity and consequently the circulation much retarded. At this juncture a physiological quantity of any potassium salt accelerates the dilatation and carries it to completion before the next contraction begins, and so the circulation is maintained.

In this paper I propose still further to develope the antagonism subsisting between these salts, and shall show that lime and potash salts are antagonistic not only in physiological but also in toxic doses.

A few years ago an interesting controversy arose respecting the mutual antagonism of drugs. On one side, Schmiedeberg, Heidenhain, Luchsinger, Langley and Prevost maintained that drugs might be mutually antagonistic, whilst Rossbach denied this statement. The former maintained that the effect of a drug might be antagonized by a second drug, which if increased in quantity would manifest its own individual effects, but these effects could be removed (or antagonized) by increasing the quantity of the first drug. To take an example from Schmiedeberg;—atropin will antagonize the action of muscarin, and muscarin the action of atropin on the heart. To take another instance from Langley;—atropin will antagonize the action of jaborandi on the submaxillary gland, and vice versâ, if after atropin has antagonized the action of jaborandi a further quantity of jaborandi is administered, it will overcome the action of atropin and salivary secretion will return.

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[1] *Journ. of Phys.* Vol. III. and IV.

Rossbach disputes this and maintains that whilst one drug increases another depresses or suspends function, and the drug which increases function can never overcome the effect of a drug that has abolished function. He admits that when atropin arrests the secretion of the sweat glands, pilocarpine may produce perspiration, but he asserts that this occurs only with a small dose of atropin just sufficient to paralyse the secretory nerves, but not the secretory cells of the gland, and that pilocarpine stimulating the cells may produce perspiration. But he maintains that a larger dose of atropin paralyses both the secretory nerves and the secretory cells, and after this stage no amount of pilocarpine will excite perspiration.

Luchsinger and especially Langley[1] have carefully and elaborately investigated this question and have established I think without doubt that drugs may be mutually antagonistic.

In this paper I hope to prove conclusively that drugs can be mutually antagonistic, and I shall show that a toxic dose of calcium chloride can be quite antagonized by a toxic dose of potassium chloride and vice versâ; that a toxic dose of potassium chloride sufficient not only to arrest spontaneous beats, but sufficient to prevent a strong induction shock exciting any contraction, can be antagonized entirely by a toxic dose of calcium chloride; thirdly, by the careful apportionment of the two salts their antagonism can be so nicely balanced, that the ventricle will beat spontaneously and quite naturally.

Moreover, in another paper I shall show that a toxic dose of potassium chloride will antagonize a toxic dose of veratria, and vice versâ, that a toxic dose of veratria will antagonize a toxic dose of potassium chloride; and again these two substances may be given with such equipoise as completely to antagonize each other so that the ventricle will beat quite naturally and spontaneously.

So that, in the case of calcium chloride and potassium chloride, and again in the case of veratria and potassium chloride, we may have in the circulating fluid two of each series present in toxic doses without either poison exerting any influence on the ventricle, which goes on beating spontaneously and normally. Yet if one salt were administered singly it would powerfully affect the functions of the ventricle to an extent incompatible with life.

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[1] For a good account of the controversy on this subject, see Langley, *Journal of Physiology*, Vol. III. p. 11.

These experiments were conducted in all respects like those recorded in preceding papers; but instead of using blood solution I employed a circulating solution consisting of 100 c.c. saline solution containing 1 c.c. of solution of sodium bicarbonate, 0·75 c.c. of potassium chloride, and 1 c.c. to 2 c.c. (according to the temperature and condition of the frog) of calcium chloride, 1 °/₀ solutions. Most of these observations were made during May and June.

Whether supplied with blood or with circulating fluid, if the ventricle ceases to act for some time, the ensuing beats whether occurring spontaneously or due to stimulation take on the "stair-case" character. The longer the ventricle remains without contracting the more accentuated the stair-case becomes. This "stair-case" character of the trace, I shall now show, depends in part on the relative amount of calcium and potassium salts in the circulating fluid.

Potassium salts favour, whilst calcium salts lessen or remove this effect of a suspension of beats on the ensuing contractions.

I. By increasing the quantity of lime in the circulating fluid this character of the trace even after a long suspension of the contractions is quite obviated.

II. If the relative quantity of potash to calcium chloride is increased either by lessening the lime or by increasing the potash salt, the stair-case character becomes still more evident. Thus if we use half the quantity of lime necessary to produce a complete contraction and a long diastolic pause ensues, the first contraction is very feeble and the contraction and relaxation very slow, the beats gradually increase in strength whilst the trace consequently rises higher and higher, the improvement of the beats being prolonged over a considerable time. On adding more lime the stair-case character becomes much less marked, or it is abolished.

III. On the other hand, if we add a potassium salt like potassium chloride to the circulating fluid we produce the same results in addition to considerable weakening of the contractions. This is well seen on adding 5 c.c. of 1 °/₀ solution to 100 c.c. of circulating fluid. On adding enough chloride of calcium solution we cannot produce the stair-case character, no matter how long the interval that the ventricle remains without contracting.

Not only are lime and potassium salts mutually antagonistic in regard to the character of the beats just described, but I shall now show that they are mutually antagonistic in respect of their influence on contractility.

If we add 10 to 15 c.c.[1] of 1% solution of potassium chloride to 100 c.c. of circulating fluid the contractions grow weaker and weaker, and at last the ventricle stops in diastole and refuses to contract on the application of a strong induction shock. On adding to the 100 c.c. of circulating fluid 1 c.c. of 10% solution of calcium chloride, contractility returns and becomes as complete as at the beginning of the experiment.

Here then we see that a toxic dose of potash sufficient to arrest contractility in the ventricle is quite antagonized by a toxic dose of lime chloride, both salts being present at the same time in the circulating fluid. In other words, two substances, each sufficient to profoundly affect the ventricle when added singly to the circulating fluid, will when added jointly in proper quantities, mutually antagonize each other so that the contractions of the ventricle are quite normal in character.

The lime we see quite obviates the poisonous effects of the potassium chloride, so far as the contractility is concerned, though in some other respects the potassium effects persist.

Faradization of the ventricle supplied with either blood or circulating fluid, increases the frequency of the contractions and induces fusion of the beats more or less complete, in proportion to the strength of the faradization, and a sufficiently strong current produces complete tetanus. On adding a potash salt to the circulation these effects are singularly modified. The fusion grows less and less, and at last in spite of very strong faradization, as with a one-celled Daniell battery and the induction coil at 0, the spontaneous beats continue quite unaffected, this strong current being quite powerless to influence the contractions. With a still larger dose of potash the faradization arrests the spontaneous contractions, which recommence on the discontinuance of the faradization. This effect of potash, i.e. the arrest of beats through faradization, I have failed to obtain in my recent experiments, but whether this discrepancy is due to the time of year or to my using circulating fluid instead of blood I am not at present able to say.

Now after a large dose of calcium chloride has obviated the poisonous effects of potassium chloride on contractility, these other effects persist; for a faradaic current which before the addition of potash and lime caused tetanus is now quite inoperative, and a current even much stronger produces scarcely any effect, the beats being only a little more frequent but not frequent enough to cause even partial fusion. Still the potash only suspends the effect of faradization, for on replacing the poisoned circulating fluid with simple circulating fluid faradization resumes its normal sway and causes fusion and tetanus.

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[1] The amount of potassium chloride required to weaken or arrest the ventricle depends on the amount of lime the circulating fluid contains; if it contains 2 c.c. to the 100 c.c. it will require more than given above.

Calcium chloride in toxic doses we have just seen will antagonize toxic doses of potassium chloride, and the same experiments of course prove that potassium chloride will antagonize calcium chloride. I propose now to describe additional experiments showing the antagonism of potassium salts for calcium salts.

A physiological dose of potassium chloride lessens the effects of even large toxic doses of calcium chloride to a considerable extent, and prevents many of the effects that occur when no potassium salt is present in the circulating fluid; hence I shall speak first of the effects of toxic doses of calcium chloride added to saline solution and then of toxic doses of calcium chloride added to circulating fluid.

When calcium chloride is added to saline solution, it delays the ventricular dilatation following a contraction; and the delay is in proportion to the amount of lime, comparatively slight with a physiological quantity but considerable with a large dose. For instance, after the addition of 3 c.c. of 10 °/₀ solution to 100 c.c. saline solution, the ventricular beat lasted thirty seconds, much of this time being occupied with dilatation.

If we first take a trace with circulating fluid, and then replace this with 100 c.c. saline solution containing 3 c.c. of 10 °/₀ solution of calcium chloride, in a short time the beat becomes enormously prolonged. After a time the contractions grow shorter and shorter in duration and slight contracture sets in, so that the whole trace rises higher above the base line.

Next the beats grow weak and very little longer in duration than with circulating fluid or blood, and at last the ventricle stops in diastole. The larger the dose of calcium chloride the quicker these changes occur, and after a large dose the duration of the beat is never so considerable as with a more moderate dose, as 3 c.c. These are the same changes, though much more marked and occurring in a much shorter space of time, as happen when a physiological dose of calcium chloride is added to saline solution.

At every stage the changes induced by lime can be obviated by a moderate dose of 1 °/₀ solution of potassium chloride. If added to the circulating fluid when the beat is greatly prolonged and therefore the trace greatly broadened, the contraction becomes quite normal. Even when contractility has ceased, the addition of potassium chloride will slowly restore contractility, which gradually increases till normal contractions return, as the following experiment shows.

I first took a trace whilst the ventricle was fed with circulating fluid and then replaced the circulating fluid by 100 c.c. saline solution containing 1 c.c. of 10 $^0/_0$ solution of calcium chloride, and in five minutes the trace of the contractions became much broader and dilatation much delayed. The trace grew broader still with fusion of the beats. It then grew weaker, till at last contractions ceased. I then added 1 c.c. of 1$^0/_0$ solution of sodium bicarbonate, which induced slight contracture and very slight contractions followed strong induction shocks: then I added 1·5 c.c. of 1$^0/_0$ solution of potassium chloride. The slight contracture soon disappeared and in about four minutes the ventricle began to beat spontaneously, the beats at first very feeble gradually improved, and in ninety minutes from the commencement of the experiments the contractions became as good as at the beginning of the experiment.

Here then with saline solution, sodium bicarbonate, and calcium chloride, the last in toxic quantities, the spontaneous beats stopped and strong induction shocks induced only very feeble contractions, but on the addition of a small quantity of potassium chloride the contracture disappeared, with recovery of perfect and spontaneous beats.

The small and physiological dose of potassium chloride (1 in 15000) in circulating fluid, greatly modifies the effect of toxic doses of calcium chloride. The addition of 3 c.c. of 10 $^0/_0$ solution of calcium chloride to 100 c.c. circulating fluid but slightly prolongs the contraction and so but slightly broadens the trace. The calcium chloride however greatly diminishes and at last abolishes excitability without weakening contractility. For instance, before the addition of the lime salt the ventricle contracted spontaneously and responded to an induction shock, the coil standing at 8: but as the lime effects intensified spontaneous contractions ceased, a stronger and stronger induction shock was needed to excite a ventricular contraction, till at last, with the coil at 0, no contraction could be induced though the ventricle contracted completely to a mechanical stimulus, and at last even this failed. The excitability however is only suspended, for on adding a further dose, as 1 c.c. of 1$^0/_0$ solution of potassium chloride to 100 c.c. of circulating fluid, excitability returned with normal contractions, the ventricle responding to an induction shock with the secondary coil at 1. Further, on replacing the dosed circulating fluid with a fresh supply of circulating fluid, the ventricle responded with the coil at 5.

Potash salts like lime salts diminish excitability, potash salts in a less degree than lime; yet we have just seen when both are added, in sufficient doses to circulating fluid, one salt does not increase the effect of the other on excitability, but the inexcitability induced by lime chloride is much diminished by the action of potassium chloride.

Here then we have an instance in addition to others I have put on record, of two drugs which severally excite the same action on a tissue; yet when administered simultaneously they do not produce the sum of their united action, but one drug in fact lessens the effect of the other.

It is hardly necessary to draw attention to this fact, as we frequently combine medicines with the intention of augmenting an effect common to each. We combine acetate of ammonia with nitrous ether or with ipecacuanha to increase perspiration; and on the other hand we give quinine, zinc sulphate, or sulphuric acid combined to check perspiration.

Now the foregoing experiments tend to show, it by no means follows, that though separately each drug acts directly on the same tissue, that in combination they will intensify their common effect.

It is true that a function may be modified in various ways. For instance, either by the direct action of the medicine on the organ, or through the central nervous system, or by means of the nerves, and we can influence function by modifying the blood supply. It is very probable that two drugs having the same property but affecting the organ through different channels may each heighten the common effect, yet I can well conceive that they may not so act. It may well happen that two drugs having a common influence, exercised in one case through its direct action on the tissue and in the second case through the nervous system, may clash and antagonize each other.

Time of year or rather temperature influences the action of remedies both in degree and kind. I have already shown that veratria acts much more powerfully in hot than in temperate or cold weather, that a dose almost inoperative in temperate weather produces considerable effect in hot weather. In cold or temperate weather, too, veratria induces only great prolongation of the contraction, but in hot weather it causes great irregularity in the cardiac contractions, due to irregularity in the rhythm of contraction, or in larger doses from incoordinate action of the ventricular muscular tissue.

Brunton, too, found that temperature influences the action of veratria on the voluntary muscles[1].

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[1] *Proceedings of the Royal Society.*

Temperature also modifies the action of potassium and especially of calcium salts on the ventricle. In hot weather they induce persistent (tonic) contraction (contracture) much more readily than in cold weather, and a dose that will not induce tonic contraction in cold weather induces a considerable amount in hot weather.

Hence the application of the antagonism, between lime and potash salts, is much more limited in hot weather, for though the antagonism as regards rapid contractility is the same at all temperatures, yet whilst we effect this antagonism, we at the same time, in hot weather, induce much tonic contraction, which effect lessens the dilatation of the ventricle and so diminishes the amount of blood it can receive and therefore propel into the arterial system, and this effect on the ventricle after a moderate, and in cold weather inoperative, quantity, is sufficient to destroy life.

Probably the condition of the muscular tissue of warm-blooded animals is more like the condition which obtains in the muscular tissue of cold-blooded animals during the hot months of the year. If this be so, then the employment of these antagonisms will be much limited in warm-blooded animals, for whilst they act antagonistically in respect of one form of contraction, they will induce another form (tonic contraction) which might be destructive to life.

[*From the Journal of Physiology. Vol. V. No. 4.*]

AN EXPERIMENTAL INVESTIGATION SHOWING THAT VERATRIA IS SIMILAR TO LIME SALTS IN MANY RESPECTS AS REGARDS THEIR ACTION ON THE VENTRICLE, ALSO SHOWING THAT VERATRIA AND ~~LIME~~ Potash SALTS ARE RECIPROCALLY ANTAGONISTIC.

By SYDNEY RINGER, M.D., *Professor of Medicine at University College, London.* Plate XII.

Some time ago[1], struck by the fact that lime and veratria salts each greatly retard the ventricular dilatation, and finding that, with regard to the lime salt, a potash salt prevents this retardation, I was led to try and ascertain whether potassium chloride would prevent the retardation arising from veratria, and found that potassium chloride answered completely.

The similarity in these respects between lime and veratria salts led me to experiment further to determine whether veratria acts similarly to lime in other particulars. In the end I find, in the following particulars, a singular similarity between these two substances.

I. In order to sustain the contractility of the ventricle lime must be present in a circulating fluid, though only in minute quantity. I had hitherto found that without a lime salt no contraction is possible. Like lime, though in a much inferior degree, veratria will sustain contractility of the ventricle. In other words, in a circulating fluid a veratria salt can replace a lime salt, though imperfectly.

II. A potassium salt antagonizes the effect of even toxic doses of lime, and vice versa, toxic doses of lime antagonize completely toxic doses of a potash salt. So it is with veratria; a veratria salt antagonizes the effects of a toxic dose of potassium chloride, and potassium chloride antagonizes entirely the effects of veratria, even when it has arrested the ventricle. Moreover the antagonism, as is the case with lime, is so complete, that after antagonizing the toxic effects of potassium chloride by veratria or of veratria by potassium chloride the ventricle beats quite naturally, though the circulating fluid contains these two substances, each of which when administered singly is sufficient to greatly weaken, or even to arrest the heart.

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[1] *Practitioner.*

In these experiments, except when the contrary is stated, I used a 0·2°/₀ solution of Morson's veratria and a 1°/₀ solution of potassium chloride, of sodium bicarbonate and of calcium chloride.

These observations were made in the summer months of 1884.

In the first place I will show that veratria, though only to an imperfect extent, can replace lime in a circulating fluid capable of sustaining the heart's contractility. To make myself better understood, I may here state that a solution containing certain quantities of common salt, sodium bicarbonate, potassium chloride and calcium chloride, employed as a circulating fluid, will for many hours perfectly sustain the heart's contractions, but, if lime is omitted, the contractility soon becomes suspended. Lime therefore in the circulating fluid is essential to the maintenance of the heart's contractility. Now I find that in an imperfect degree veratria can replace lime.

I first took a trace whilst the ventricle was fed with circulating fluid[1]. Then I replaced this by 200 c.c. saline solution containing 2 c.c. sodium bicarbonate, 1·5 c.c. of potassium chloride and 0·5 c.c. of veratria solution. The ventricular contractions then rapidly grew weaker and the contractions soon assumed a characteristic veratria character. On adding 1·3 c.c. of potassium chloride solution the weakened beats improved in strength, and the spontaneous contractions, though registering not a quarter so high as at the beginning of the experiment when the ventricle received circulating fluid, persisted an hour and a quarter under the influence of the veratria solution. I then replaced this veratria solution with circulating fluid. The contractions improved, but at first maintained the veratria character still more distinctively: then the veratria character of the beat gradually became less and less evident, and at last the beats became normal.

This result however I did not obtain in every instance, for in some cases the contractions grew weak and soon ceased.

If a fluid is used of the same composition as the veratrized fluid minus the veratria the contractions speedily grow weak, soon stop, and induction shocks fail to excite contractions, showing that when the verātria is present the persistence of the contractions is due to this agent.

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[1] The circulating fluid spoken of in these experiments consisted of 100 c.c. saline solution containing 1 c.c. of sodium bicarbonate, 1 c.c. of calcium chloride and 0·75 c.c. of potassium chloride solutions.

The effect of veratria as a substitute for lime is however better shown in another way.

I supplied the ventricle with the following circulating fluid:—300 c.c. saline solution with 3 c.c. of sodium bicarbonate, 2 c.c. of potassium chloride, and 1 c.c. of calcium chloride solutions; that is with a mixture containing only a quarter the quantity of lime necessary to maintain good contractions in the summer months, especially if the frogs are weakly.

The beats soon grew weak. At this stage I added 1 c.c. of veratria solution and the contractions soon became strong.

This result I have obtained in many cases. Here then we see that veratria strengthens a ventricle weakened by a too scanty supply of lime and the ventricle is made to contract fully. The veratria indeed acts as a substitute for lime.

I have already shown that a reciprocal antagonism exists between lime and potassium salts, and now I will proceed to show that there is a reciprocal antagonism between veratria and potassium chloride.

Potassium chloride quite obviates the effect of a poisonous dose of veratria and restores strong and natural contractions, and vice versa, veratria restores perfectly natural beats to a ventricle arrested by potassium chloride.

In the first place I will give instances of the nullification of the effects of veratria by means of potassium chloride. A moderate dose of veratria, as for instance 1 c.c. of 0·2°/$_0$ veratria solution to 200 c.c. of circulating fluid, produces great irregularity of the cardiac contractions[1], which toxic effects a moderate dose of potassium chloride entirely removes and restores quite natural beats.

I first fed the ventricle with a circulating fluid composed of 200 c.c. of saline solution containing 2 c.c. of sodium bicarbonate, 1·5 c.c. of potassium chloride and 1 c.c. of calcium chloride solution, and then added to the circulating fluid 1 c.c. of veratria solution. The veratria strengthened the contractions considerably (see A. Fig. I. Pl. XII) and next produced great irregularity in the contractions, see B. I then added

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[1] This irregularity is first one of rhythm and strength, the irregularity in strength depending, as I shall subsequently show, on the irregularity in rhythm. At a later period incoordination of the ventricular muscular tissue comes on, through one part acting out of unison with the rest.

3 c.c. of potassium chloride solution, which quite neutralized the veratria effects and induced normal contractions, see B. and C.

We have then an instance of the circulating fluid containing a poisonous dose of veratria and a dose of potassium chloride greatly in excess of the physiological quantity, and yet, from their mutual antagonism, the ventricle remains altogether unaffected by either, and perfectly normal beats remain.

Larger doses of veratria greatly weaken or even arrest the ventricle in diastole, and these effects a dose of potassium chloride, toxic per se, will quite neutralize. In the following instance the veratria greatly weakened the contractions. Hereafter I shall have occasion to describe an experiment where veratria arrested the ventricular contractions entirely.

I first took tracings under circulating fluid and then added 1 c.c. of 0·5 °/₀ solution of veratria to 100 c.c. of circulating fluid; this at first increased the strength of the contractions, then induced irregular action and next greatly weakened the contractions (Pl. XII.), see A. Fig. II. The addition of 10 c.c. of potassium chloride solution removed the veratria effects and restored good natural contractions (see B).

I next go on to show that, like lime, veratria antagonizes a poisonous dose of potassium chloride. First, taking a tracing of the contractions, the ventricle being supplied with circulating fluid, I then added 15 c.c. of 1 °/₀ solution of potassium chloride to 200 c.c. of circulating fluid. The contractions soon grew weaker, whilst the height of the trace became greatly reduced.

I then added some veratria solution 0·5 °/₀, little by little. After adding 2 c.c. good spontaneous contractions returned, as good almost as at the beginning of the experiment with circulating fluid simply. The contractions were quite normal, neither the contraction being prolonged nor the dilatation delayed. I then added another cubic centimetre of veratria solution and the beats grew much feebler, being reduced to one-third the size of the beats previous to the last addition of veratria, but the beats still remained quite free from any veratria character.

Here then veratria strengthened a ventricle almost arrested by potassium chloride, but the potash prevented the veratria from developing its characteristic modification of the beat, even though the veratria was added in a dose sufficient to greatly weaken the ventricle.

I repeated this experiment in a modified way, as may be seen in the following account.

I first took a trace of the ventricle supplied with 200 c.c. of circulating fluid (see A. Fig. III.), and then added 14 c.c. of 1 °/₀ solution of potassi chlorid and 1·75 c.c. 0·5 °/₀ solution of veratria. The ventricular contractions at first became more rapid, rather weaker, and a little irregular in height. The contractions then grew slower and stronger.

Then the addition of another 0·5 c.c. of veratria solution gave trace B. Fig. III., the contractions being as good though less frequent than with circulating fluid. Here then these two substances, potassium chloride and veratria, though each separately in sufficient quantity to completely arrest contractility in the ventricle, were almost inoperative, the one antagonizing the other.

I then replaced the poisoned circulating fluid by 200 c.c. fresh circulating fluid. In two minutes the trace showed well-marked veratria effects, which gradually disappeared, and in six minutes the trace became quite normal. I then added 14 c.c. of potassium chloride solution, the same quantity employed when mixed with veratria, this quickly weakened almost to extinction the ventricular contraction, see C.

Next I added 2·25 c.c. of 0·5 °/₀ veratria solution, the contractions quickly improved and in twelve minutes the ventricle gave trace D.

Here then potash almost stopped the ventricular contraction and veratria restored good contractions. Again I replaced the poisoned circulating fluid by 200 c.c. of circulating fluid, and as before the heart's trace showed well-marked veratria effects, proving that potash having a higher diffusion or a weaker affinity for the tissues than the veratria is removed more quickly from the tissues of the heart. The veratria effect soon disappeared. I then added to the 200 c.c. circulating fluid 2·5 c.c. of veratria solution and obtained the effect recorded in trace E. Fig. III. This dose of veratria then well-nigh arrested the ventricular contractions, reducing them to a wavy line. I then added 14 c.c. of potassium chloride solution, which quickly improved the beats, but only to rather more than a third of the strength at the beginning of the experiment. It struck me that the ventricle with all this varied treatment must be weakened and that possibly, under these circumstances, the potash might take more effect. So I again supplied the ventricle with a fresh 200 c.c. of circulating fluid, and again at first well-marked veratria effects appeared, which however passed off and the contraction became quite normal. I then added 2 c.c. of veratria solution to the 200 c.c. circulating fluid and soon the traces became reduced to a wavy line, the ventricle being well-nigh arrested. I then added 5 c.c. of potassium chloride solution, which quickly strengthened the beats, as is seen in trace F. Fig. III. Some veratria effects remaining, I added 2 c.c. of potassium chloride solution and produced good contractions, see G.

In this experiment, the simultaneous addition of both veratria and potassium chloride in toxic doses produces very little effect on the trace; but the same dose of potassium chloride administered alone all but arrests the ventricle, whilst the addition of the same dose of veratria δ/ obviates the effect of the potash salt~~s~~ and restores good contractions to the ventricle. Further, on replacing the poisoned circulating fluid by a fresh supply of circulating fluid, then adding the same dose of veratria, the ventricle undergoes arrest in diastole, but the addition of the same salt dose of potash ^ neutralizes the veratria effects. Potassium chloride and veratria are therefore reciprocally antagonistic. In the first part of this experiment the circulation contained potash enough or veratria enough when administered singly to arrest the ventricle, but administered together they remain inoperative.

Temperature modifies the antagonism between veratria and potassium chloride, as it does the antagonism between calcium and potassium salts and the antagonism of atropia for aconitia.

Whilst in hot weather potassium will still completely antagonize veratria, veratria on the other hand only partially antagonizes a large dose of potassium chloride; and a much smaller dose of veratria must be used, for the dose adequate in cool weather acts so powerfully that it weakens or arrests the ventricle.

EXPLANATION OF FIGURES. PL. XII.

Fig. I.

Shows the effect of a dose of veratria insufficient to arrest the ventricle, but sufficient to produce extreme irregularity of the rhythm and force of the beat and marked incoordination.

A. Shows the strengthening effect of veratria on the ventricular contractions: the veratria was added at the point indicated by an arrow.

B. Shows the effect of adding 1 c.c. of 0·2 $^0/_0$ solution of veratria to 200 c.c. of circulating fluid, and also the antagonizing effect of 3 c.c. of 1 $^0/_0$ solution of potassium chloride, which were added at the point indicated by an arrow.

C. Shows the effect of the potassium chloride twelve minutes later than the preceding trace.

Fig. II.

A. Shows the effect of 1 c.c. of 0·5 veratria solution added 40 seconds before the beginning of the trace to 100 c.c. circulating fluid. At the point indicated by an arrow 10 c.c. of 1 °/₀ solution of potassium chloride were added, which quickly antagonized the effect of the veratria.

B. Shows the trace twelve minutes after the addition of the potassium chloride.

Fig. III.

A. Trace taken with artificial circulating fluid.

B. After adding to 200 c.c. of circulating fluid 14 c.c. of 1 °/₀ solution of potassium chloride and 2·25 c.c. of 0·5 °/₀ solution of veratria.

C. Trace with circulating fluid. 14 c.c. of potassium chloride solution were added at the point indicated by an arrow.

D. After the addition of 2·25 c.c. of veratria solution.

E. Showing the effect of 2·5 c.c. of veratria 0·5 °/₀ solution to 200 c.c. circulating fluid at the point indicated by an arrow.

F. Showing the effect of adding to the above solution containing veratria 5 c.c. of potassium chloride solution.

G. Showing the effect of the further addition of 2 c.c. of potassium chloride solution.

Plate XII.

Figure I.

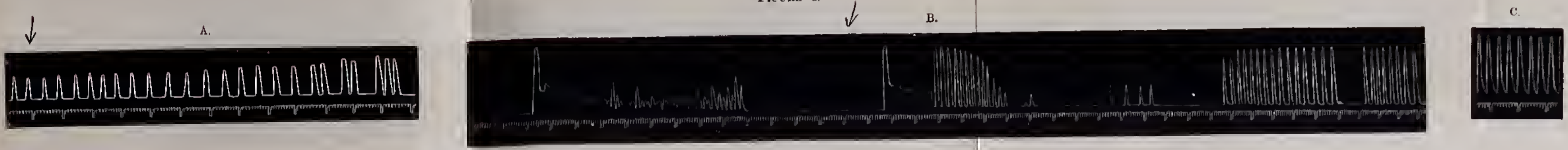


Figure II.

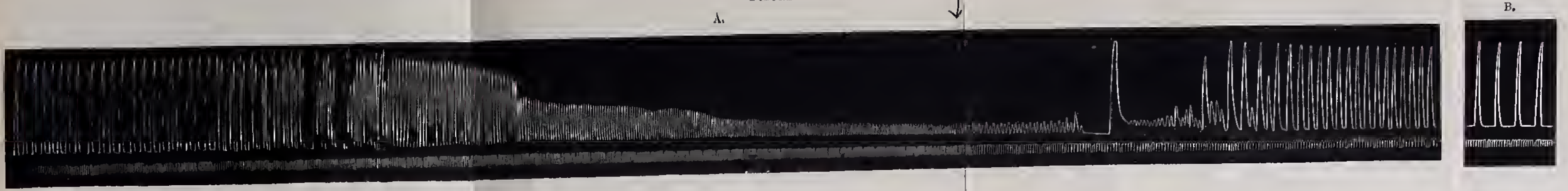
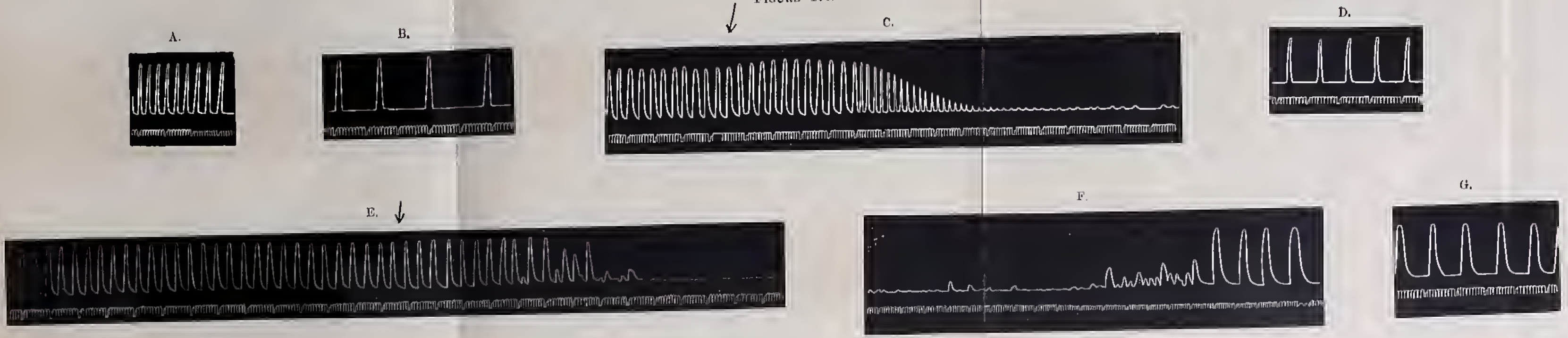


Figure III.



[*From the Journal of Physiology. Vol. VI. No. 4.*]

CONCERNING THE ACTION OF SMALL QUANTITIES OF CALCIUM, SODIUM, AND POTASSIUM SALTS UPON THE VITALITY AND FUNCTION OF CONTRACTILE TISSUE AND THE CUTICULAR CELLS OF FISHES.

By SYDNEY RINGER, M.D., F.R.S., *Professor of Medicine in University College, London,* AND DUDLEY W. BUXTON, M.D., B.S., M.R.C.P., *Assistant to the Professor of Medicine in University College, London.*

In previously published papers we have shown that very minute quantities of Potassium and Calcium Salts possess the power of influencing the contractility of the heart. We have pointed out that even so minute a quantity of Potassium chloride as 1 in 15,000 and of Calcium chloride as 1 in 10,000 is capable of promoting the normal cardiac contractility provided they are brought into contact with the heart ventricle in the presence of a simple [$\frac{3}{4}$ %] saline medium. We do not even attempt to explain in this place how such minute quantities of inorganic salts can influence so profoundly the organised structure of the heart. However, it may be said that it does not seem improbable, that the salts do not enter into direct combination with the molecules of the muscular tissue; and further that if any combination exists it is of an extremely loose character since the salts are easily dislodged and washed away by the mere circulation of a fluid devoid of these salts.

But it appears necessary, in order that contraction should take place, that minute quantities of these salts should be present in the fluid environing the muscular tissue. The same condition also obtains for plants. Thus we read: "Whilst carbon, hydrogen, oxygen, nitrogen and sulphur mainly comprise the organised parts of a plant,—potassium, calcium, magnesium and iron are of less importance if only in consequence of their much smaller quantity; they appear to promote chemical decomposition and combination in plants in consequence of which the far more abundant combustible principles are constructed out of the first named five elements.[1]"

We thought that since extremely small quantities of these salts when in contact with muscular tissue produce remarkable effects, and affect in some way the molecular changes occurring during contraction, that it would be well to ascertain how distilled water would behave toward protoplasm. Distilled water being purely negative as regards salts would we should expect either be passive or possibly possess an action which would be more or less masked in the presence of salts. To test the behaviour of distilled water we placed Fish in vessels containing it. It occurred to us that we should, by disturbing the equilibrium of diffusion between the circumambient fluid on the one hand and the blood of the fish as well as the parenchymatous fluids of the surface cells of the fish on the other, influence the life of the fish. Our experiments substantiated the justice of our conclusion for fish placed in distilled water died in a few hours. When however minute quantities of salts of calcium or potassium or sodium were added the life of the fish was prolonged. The lime salts were found to possess the greatest power of sustaining life. It was further elicited that fish can be habituated to live in distilled water provided minute quantities of lime are present[2].

The question, however, yet remained unsolved, why the fish rapidly died when placed in distilled water. To solve this difficulty we sought to discover whether the distilled water caused the lime and other salts contained in the tissues of the fish, to diffuse out. If so, and these salts were absolutely necessary to protoplasm in order that it should perform its vital functions, it would readily be understood that upon the abstraction of the salts the tissues would cease to be able to fulfil their function and so would die. But, it was also possible that placing the fish in distilled water disturbed the equilibrium of diffusion between the surrounding water and the cells covering the gills and so caused salts to diffuse out of the cells and water to pass into the cells until they became irreparably damaged and disorganised. In the paper cited above these questions were discussed, but it was at that time impossible to arrive at any definite result and hence the answer was then left open. We subsequently undertook the elucidation of these points in the investigations which form the subject of the present communication.

[1] Sachs, *Treatise on Botany*, 2nd ed., p. 696.

[2] *Journal of Physiology*, vol. v., no. 2.

The method employed may thus be briefly summarized. The gills of the fresh-water Mussel [Anodon] were carefully cut so that the edge bearing the cilia was rapidly removed, care being taken to avoid injury or exposure. The gills were then divided as nearly as possible into equal portions and placed in the solutions. In every case the series were arranged so that only portions of the gills of one Mussel were employed, and hence were strictly comparative. The solutions were composed of distilled water, either alone or with solutions of salts of 1°/₀ strength. In each experiment 400 c.c. of fluid were employed. As the gills might be supposed to part with substances favourable to the sustenance of vitality when left in distilled water, just as we have shown is the case with tadpoles and fish, we changed the solutions in which the gills were placed twice in every twenty-four hours. The importance of this precaution will be shown in the sequel, since the presence of even a minute quantity of lime in the fluid might tend to sustain ciliary action. The portions of gills were examined every day under a high power of the microscope, the specimen being in each case investigated in its own medium. The action of distilled water upon the ciliary apparatus and movements of the cilia of the gill edge may first be recounted. Six observations showed that ciliary action always ceased within twenty-four hours. The cells of the gill edge presented a curious appearance. They were separated from the gill edge and had lost their sharp outline, having become swollen up to three or four times their natural size and further being considerably distorted in shape. The cells looked markedly granular and were in different stages of disintegration. In some instances this disintegration had gone so far that only the nuclei were left and even they were granular. In some preparations it was thought that a distinct swelling up of the intercellular substance could be made out, thus separating the cells from one another. We then tested the effect of adding calcium chloride to the distilled water.

In four experiments in which we used 2 c.c. of the 1°/₀ solution of calcium chloride to every hundred distilled water, i.e. 1 in 5000, we found that ciliary action had in the case of three of the specimens disappeared in twenty-four hours while in the fourth it had almost ceased. The tissue of the gill had further become greatly disintegrated.

In four experiments in which we added 4 c.c. of calcium chloride solution to the hundred distilled water, i.e. 1 in 2500, the ciliary action in one had disappeared in 48 hours and in the second in 72 hours, while in the third, ciliary action ceased on the 4th day, and in the fourth upon the 5th day.

We also employed still larger quantities of lime; thus in one experiment working with 8 c.c. of calcium chloride solution to the 100 c.c. of distilled water, i.e. 1 in 1250, it was found that ciliary action persisted to the 7th day.

With 16 c.c. of calcium chloride solution, i.e. 1 in 625, two experiments were undertaken with the result that in one the ciliary action persisted on the 7th and in the other on the 8th day. It would then appear that while distilled water rapidly causes ciliary action to cease that calcium chloride prolongs the life of the cilia and greatly retards the disintegration of the tissue.

Sodium bicarbonate also when added to distilled water sustains the life of the cilia, so that ciliary action persists, and sodium bicarbonate checks the disintegration of tissue which we found occurs with distilled water alone. These statements are based upon the following experiments.

When 1 c.c. of sodium bicarbonate solution to the 100 c.c. distilled water, i.e. 1 in 10,000, was employed, ciliary action ceased in 48 hours. In five experiments with 2 c.c. of sodium bicarbonate solution to the 100 c.c. distilled water, i.e. 1 in 5000, in one, ciliary action was slight in 24 hours, in two it had disappeared in 24 hours and in two in 48 hours. Four experiments were instituted in which 4 c.c. sodium bicarbonate solution were added to the 100 c.c. distilled water i.e. 1 in 2500 and then we found ciliary action disappeared on the 4th, on the 5th in two; persisting in the remaining two experiments on the 5th and 6th days respectively.

When 8 c.c. of the sodium bicarbonate solution per 100 c.c. distilled water, i.e. 1 in 1250, were used, ciliary action persisted on the 7th day: but in two experiments in which double the above strength was employed the ciliary action disappeared in one case on the 7th in the other on the 2nd day. These experiments bring out a further point than those noticed above, and that is, that both in the case of the lime salt so also with the sodium salt, increasing the dose of the salt increases also the duration of the persistence of the ciliary action by sustaining the life of the cilia.

In the next series of experiments we determined the action upon the cilia of mixtures of salts in the test solutions.

1 c.c. each of calcium chloride and sodium bicarbonate solution were added to the 100 c.c. distilled water, i.e. 1 in 10,000 of each, and in two

experiments the ciliary action had ceased in 48 hours. Eight experiments were carried out in which 2 c.c. of the above solutions were added to the 100 c.c. distilled water, i.e. 1 in 5000 of each, with the following results: in one ciliary action ceased in 48 hours; in one it was good in 48 hours; in one ciliary action persisted on the 4th day and in three on the 5th day; in one on the 6th and in one on the 7th day respectively.

Working with double these quantities we found in one experiment (4 c.c. of each to the 100 c.c. distilled water) ciliary action had not ceased on the 7th day. When 8 c.c. of a solution of each of the above salts to the 100 c.c. distilled water were tried, in two experiments the action of the cilia still was present on the 7th and 8th days respectively.

We were thus led to the conclusion that a mixture of these salts in the test fluid possessed the power of sustaining the vitality of the cilia, and of checking the disintegration of the tissue in a higher degree than either of the salts calcium chloride or sodium bicarbonate when taken alone.

In our further experiments with solutions containing several salts, potassium chloride was added to the calcium chloride and sodium salt. Thus in two experiments 2 c.c. of the calcium and sodium salt solution and ·5 c.c. of the potassium salt solution were added to the 100 c.c. of distilled water, with the result that in one case ciliary action ceased on the 3rd day, while in the other it persisted on the 7th day. In the next two experiments the same proportions of the calcium and sodium salts were used, but the potassium salt was doubled. In one instance the ciliary action ceased on the 3rd, and in the other on the 4th, day.

We further used the New River Company's tap water, the composition of which is

Calcium	38·3	per million,
Sodium	23·3	„
Potassium	7·1	„

and other constituents which do not interest us in this connexion; and as a result of six experiments we found ciliary action persisted in one case on the 3rd, in one on the 4th, and in four on the 5th day. At this time all the gill was used up and hence the experiments were perforce brought to a conclusion.

We may then infer from the above experiments that while distilled water causes ciliary action rapidly to cease, that calcium chloride and sodium bicarbonate act in the other direction and prolong the period of activity of the cilia, the sodium salt being perhaps the more efficacious of the two. It also appears that combinations of these salts even in smaller proportions are more effectual than sodium bicarbonate when alone.

With regard to the disintegration of tissue which accompanies the use of distilled water or of weak saline solutions, it appears to us to be of a purely physical nature and will, we submit, lend itself to the following explanation.

Diffusion must under the conditions of the experiment be rapid, and being wholly from the tissue of the gill to the surrounding medium, would determine a corresponding osmotic current from the fluid to the cells of the gill. There must be as a consequence of this a swelling up of the cells, and finally bursting and disintegration of the cells. The presence of salts would obviate such a physical change, and by protecting the gill tissue enable the cilia to retain their normal activity.

If we now consider the question of the composition of the cells which line the gills of fish, we shall see that both they and the cells of the cuticular covering of fish as also the blood itself, must vary as regard the quantity of the salts they contain, for diffusion, determined by the nature of the surrounding medium, would occur until an exact equilibrium between the salts in the tissues and those in the medium had become established. Thus it becomes readily comprehensible that great disturbance in the animal economy of fish must ensue upon even slight variations in their environing fluid. And the same would obtain where the fish passed from one medium to one of a different saline composition. When fish therefore leave the sea to travel to the fresh water rivers, they usually perform their journey slowly. The slowness of their passage allows of a gradual production of the inevitable equilibrium of diffusion and so avoids the fatal disturbance which must have ensued had the change been suddenly effected. In the opposite direction, the passage from the river to the sea, the change would require the same caution, since it predicates the necessity for a converse change, that is diffusion into the cells and osmosis from them. The disturbance which occurs in either case must be largely influenced by the osmotic equivalent of the salt which diffused. Sodium chloride possesses, we should remember, a low osmotic equivalent and would hence affect the fish less than other salts of higher osmotic equivalent. Lime salts on the other hand have an osmotic equivalent ten times as great as that of sodium chloride and so would, quantity for quantity, affect the fish far more. Again the rate of diffusion and osmosis depends upon the amount of salt on each side of the membrane separating the fluids under observation. Thus in the case of the cells of fishes, especially the delicate lining of the gills, the same law would tend to modify the rate of osmosis and diffusion through them, and indeed throughout the whole body of the fish. It further is necessary to take into consideration the effect of pressure upon osmosis. By sufficiently heightening the pressure you may lessen or prevent osmosis, while diffusion remains unaffected. So, we may easily appreciate the importance of the slowness of the migration of fishes from sea to fresh water. For we see on the one hand the slowly and hence unobnoxious diffusion equilibrium established, and on the other the pressure exercised by the cell wall moderating osmosis. Thus is it that those physical influences which produce cell changes in fishes subjected to alteration in their habitual environment, may through the delayed progress of the fishes have their deleterious effects upon the fish reduced to a minimum.

The slow rate which characterises the migration of most fishes, need not, we think, be set down solely to an instinct. We would suggest that a purely physical explanation exists which will in the main account for the behaviour of fish during their migration. For, distilled water dropped upon the conjunctiva causes smarting. It gives rise to pain if applied to the nasal and probably to any mucous membrane, doing so by setting up osmotic action in the nerve terminations. If now small quantities of salts are added to the distilled water no smarting occurs. But when the salts are added above a certain quantity, smarting again is felt. Now we submit that the fish when passing to or from one medium to another more or less saline, become subjected to the full force of such osmosis-producing conditions, they would feel the brunt of the resulting effects directly as the rapidity with which they accomplished the change. Hence these inimicable conditions would determine for them a slow journey, since by delay they escape pain and deleterious influences.

We have yet to consider the difficulty which may be held as telling against the above suggestion, viz. that some fish resist the baleful effect of distilled water or an impoverished saline medium better than others; and that some fish are competent to pass with great rapidity from one medium to another and do so without manifesting any great sign of distress. Thus we ourselves found that sticklebacks would survive minnows several hours when placed in the distilled water.

We think it may be substantially urged that, as we have pointed out above, the influence of pressure here manifests itself. The cells of the fish, and especially those covering the gills, may vary in the strength of their cell walls, and in proportion to the strength of this wall so will be the osmosis:—when the pressure is sufficiently great the osmosis will be in abeyance, diffusion alone being at work.

In conclusion then we think our experiments show that distilled water is actually harmful to protoplasm: that it not only prevents the due performance of its function, but induces its actual destruction. And next that additions of minute quantities of salts obviate the destruction of protoplasm, while if present in adequate combination they maintain protoplasm so that it can perform work. As with the heart so with the gills, some salts effect this purpose better than others. We also show that the life and due performance of vital functions of animals, i.e. protoplasm in a state of high differentiation, is in the case of fishes dependent upon the medium in which they exist. Further we attempt to explain how this medium plays a part so important in the life-history of the fish.

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AN EXPERIMENTAL INVESTIGATION TO ASCERTAIN THE ACTION OF VERATRIA ON A CARDIAC CONTRACTION. By SYDNEY RINGER, M.D., F.R.S., *Professor of Medicine at University College, London.* Plate II.

In a muscular contraction three distinct forms of molecular change are obvious; namely, the changes occurring during the latent period; the changes which ensue during contraction, and the changes causing the dilatation.

Those molecular changes which lead to dilatation are generally considered restorative or reparative; they prepare new material for the ensuing contraction or remove waste products which would retard or prevent contraction; or they consist of both these processes combined.

With a skeletal muscle, it is evident that two sets of changes occur during relaxation—changes which permit relaxation and changes which prepare the muscle for a subsequent contraction. These changes can be partly dissociated, for with a fatigued muscle the amount of the contraction, the strength of the stimulus remaining the same, depends greatly on the interval between the contractions. If the interval is short the contraction is weak; if the interval is long the contraction is much stronger, and its strength is in proportion to the length of the interval between the contractions.

As might be expected cardiac muscle undergoes the same series of change, and in this paper[1] I shall show that the reparative changes, which prepare the muscular tissue for another contraction, can by various means be delayed and more or less dissociated from dilatation. I shall show that veratria retards these reparative changes to such an extent that the dilatation may be complete, but if another contraction immediately follows it will be very weak, so weak indeed that it may be scarcely evident, either by the eye or by the graphic method; and I shall show that the strength of the contraction is in proportion to the diastolic pause, and that in some cases, this must persist for a minute before the completion of the reparative changes necessary to produce a perfect contraction.

Opium and morphia and potash and other salts act in the same way, though in a less degree. Exhaustion likewise retards these reparative changes, dilatation remaining unaffected.

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[1] These experiments were made with Roy's tonometer and the ventricle was fed with a circulating fluid composed of 100 c.c. saline solution, ·6 $^0/_0$ containing 1 c.c. of sodium bicarbonate, 1 c.c. of calcium chloride, and ·75 c.c. of potassium chloride 1 $^0/_0$ solutions.

Veratria causes irregularity in rhythm, and irregularity in force of the contractions through its effect on rhythm. The earliest effect is the occurrence of two contractions, one quickly following the other, and then occurs an interval, very variable in duration, again followed by two beats in quick succession. The strength of the contraction and its duration are in proportion to the diastolic interval. The second of the two contractions which occur close together, is often much weaker than the first when it is preceded by a long diastolic pause.

Fig. 1, Pl. II. shows this influence of veratria in a ventricle only very little affected by it. The irregularity of rhythm is very slight, the difference in time between the beats being very small.

Every alternate beat is stronger than the intermediate beat, and it is evident that the duration of the contraction is in proportion to the diastolic pause preceding it.

When the effect of veratria is more marked, the dependence of the strength of the beat on the length of the diastole becomes still more apparent, as is shown in Figs. 2 and 3. In both these traces the contractions were much reduced in strength, but after a long diastolic pause the ensuing beat became much stronger.

Indeed this influence of veratria is often still more marked, as Fig. 4 shows.

Here the ventricle beat frequently and the trace was reduced to a wavy line; (see A). The contractions then ceased entirely for about a minute and there occurred the strong contraction shown in B; but this was followed by frequent contractions permitting of very little reparative change, and consequently the beats were again reduced to a wavy line and indeed are scarcely distinguishable in the trace.

We see then that the amount of the contraction in a ventricle poisoned with veratria depends on the length of the diastole. If the dose is large then the reparative changes are so slowly performed that a long diastole is necessary to the production of a contraction of normal strength. In the early stages of veratria poisoning, the rate only (rapidity not the amount) of these reparative changes is affected, and provided the diastole is sufficiently prolonged, the ensuing beat will be strong and complete.

Potash salts also lessen the reparative changes, whilst at the same time they greatly diminish the strength of the contraction no matter how long the diastole lasts; see Fig. 5. A gives the contractions when the ventricle was supplied with circulating fluid. At B is seen the effect of 15 c.c. of 1% solution of potassium chloride added to 200 c.c. of circulating fluid. The beats are weaker and their strength depends on the duration of the diastole, although even when this is prolonged, the contraction is feebler than with the unpoisoned ventricle. Potash salts then both delay restoration and lessen the amount of contraction. This diminution in the amount of contraction may be due to diminution in the amount of restoration or it may be due to potash salts depressing the conditions that favour the molecular changes undergone during a contraction. If potash salts act by lessening the amount of reparation, then a potash salt is an example of a drug that not only retards, but lessens the amount of reparation.

Morphia and extract of opium in large doses retard the reparative changes whilst they also somewhat weaken the amount of contraction.

With a weak heart as of an exhausted sickly frog kept for instance in the tanks for some time in hot weather, I find the reparative changes delayed; see Fig. 6. In this frog, the ventricle was very weak and did not completely contract. The contractions were all excited by induction shocks. The trace is much like that given by a veratrized heart. If the interval between the contractions is short, then the ensuing contraction is weaker; but if the interval is longer, then the following beat is much stronger. Trace B was taken fifty minutes after A.

The independence of those molecular changes which occur at the termination of a contraction (and which cause dilatation) from the reparative changes is well shown by the action of lime on the ventricle. Calcium chloride, added to saline solution, greatly delays dilatation, but does not in any degree delay the reparative changes, so that no matter at what period the excitation is made, the succeeding contraction is complete, even though the excitation is applied before dilatation is finished. Here then we have a drug which like veratria retards those molecular changes that lead to dilatation, but unlike veratria in no degree lessens the rapidity of the reparative changes.

What is the nature of the changes occurring during the reparation? Are they constructive whereby material is prepared for a subsequent contraction? Or are waste products which prevent or lessen contraction removed? Or do both these processes take place? These experiments suggest that the reparative changes consist in part at least in the removal of products prejudicial to contraction.

We have seen that under the influence of veratria the strength of the contraction is in proportion to the preceding diastolic pause: but this is not the only condition which regulates the strength of the contraction, for, given the same length of diastolic rest, the strength of the subsequent contraction depends on the strength of the preceding contraction. If a contraction is feeble then the products are less and their removal requires a shorter time, whilst if the contraction is strong the products of destruction being greater require a longer time to get removed. So that the greater the contraction, the weaker the subsequent contraction, the diastolic pause being the same.

We have seen that both veratria and potash salts retard the reparative changes, veratria more so than potash, and yet when these substances are both added to the circulating fluid they do not in this respect intensify each other's action, but distinctly antagonize each other, the reparative changes occurring much more rapidly after the addition of potash to the veratrized circulating fluid. We have then another instance of drugs having a common action; and yet when both are conjointly added to the circulating fluid one antagonizes the other in respect of this common effect.

Veratria then affects a muscular contraction in various ways. It has but little effect on contraction, rather favouring it, but it greatly delays dilatation and protracts the beat. It often causes irregularity of rhythm, and after large doses to a very marked extent. It greatly retards reparation, and as the beats occur at irregular intervals, the strength of the contractions vary accordingly. Further after large doses veratria induces marked incoordination of the muscular tissue; some portions are contracting whilst other portions are dilating.

EXPLANATION OF PLATE II.

Fig. 1. Early effect of a small dose of veratria, affecting rhythm, and so affecting the strength and duration of the contraction.

Figs. 2 and 3. More marked action of veratria.

Fig. 4. A shows the effect of veratria on a ventricle beating frequently. The contractions were so weakened that the trace is reduced to a wavy line. The contractions then ceased for about a minute and then there occurred a strong contraction shown in B, again followed by frequent contractions scarcely visible in the trace.

Fig. 5. A. A trace of the ventricle fed with circulating fluid.

B. A trace after the additions of 15 c.c. of $1^{\circ}/_{0}$ solution of potassium chloride to 200 c.c. of circulating fluid. Each contraction is due to an electric stimulation.

Fig. 6. Weak Heart of sickly frog; contractions induced by repeated induction shocks.

Fig. 1.

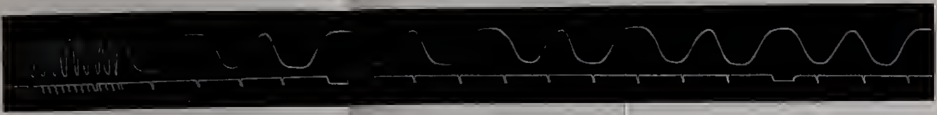


Fig. 2.

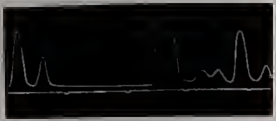


Fig. 3.

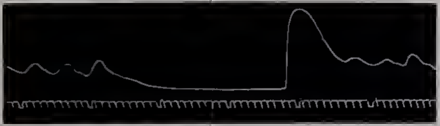
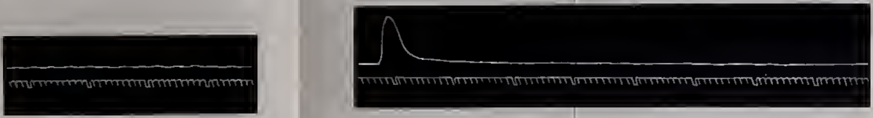


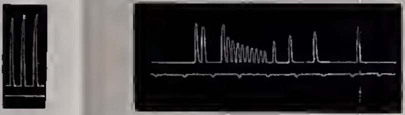
Fig. 4.



A.

B.

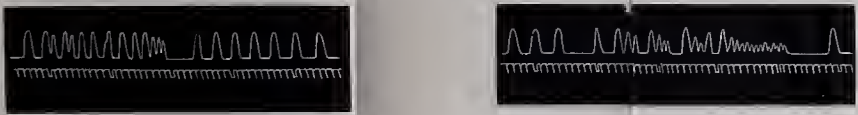
Fig. 5.



A.

B.

Fig. 6.



A.

B.

REGARDING THE INFLUENCE OF THE ORGANIC CONSTITUENTS OF THE BLOOD ON THE CONTRACTILITY OF THE VENTRICLE. By SYDNEY RINGER, M.D., F.R.S., *Professor of Medicine at University College, London.* (Pl. IX.)

PART I.

Kronecker[1] and his pupils Martius and Von Ott stoutly maintain that the frog's heart does not feed on its own substance, and that as soon as nutritive fluid is withdrawn its contractility ceases. They further assert that only fluids containing serum-albumin are adequate to sustain the heart's contractility.

In common with other observers they find that if a ventricle is fed with ·6 $^0/_0$ saline solution contractility soon ceases, and a strong induction shock will not excite a contraction. They conclude that the suspension of contractility depends on the saline solution washing out all nutritive material from the heart's cavity and the meshes of its spongy substance.

Merunowicz[2] however finds that the dissolved ash of incinerated blood furnishes a good fluid for supporting the heart's contractility, and Gaule[3] concludes that the essential agent in the ash which sustains the contractility is an alkaline carbonate. Like subsequent observers he too finds that the addition of an alkaline sodium salt, even in small quantity, to saline solution enables it to sustain contractility longer than when simple saline solution is used. This fact leads him to differ from Kronecker, and he avers that the heart can feed on its own tissues and derive from them the materials required for a contraction. Martius objects to this conclusion, and maintains that alkaline solution containing caustic soda may act by combining with the carbonic acid formed by the contraction and thus save the muscular tissue from asphyxia.

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[1] Martius. Du Bois-Reymond's *Archiv.* 1831, p. 474. Kronecker and Von Ott. *Ibid.* p. 569. Martius. *Ibid.* 1882.

[2] *Arbeiten aus der Physiologishen Anstalt zu Leipzig.*

[3] Du Bois-Reymond's *Archiv.* 1878, p. 291.

In several papers published in this Journal I have shown that the addition of a lime salt to saline solution will prolong the period of contractility to a much greater extent than an alkaline sodium salt, but the character of the contractions undergoes much alteration. Further, I have shown that the addition to the circulating fluid of a physiological quantity of a potassium salt will entirely obviate these alterations, and that this addition adds greatly to the duration of contractility. Indeed a fluid consisting of 100 c.c. of ·6 % sodium chloride solution containing 1 c.c. of sodium bicarbonate, 1 c.c. of calcium chloride, and ·75 c.c. of potassium 1 % solutions will, in some months, sustain contractility for one or two hours, and even at the end of three hours with the ventricle of a well-nourished frog I have found the contractions as good as at the beginning of the experiment.

In an experiment made in July I found that a saturated solution of phosphate of lime in distilled water containing ·6 % sodium chloride and one part in 10,000 of sodium bicarbonate, and one part in 20,000 of potassium chloride, sustained good contractions for three hours and a half, when the experiment was discontinued.

On July 9, a ventricle in Roy's apparatus, fed with saline solution saturated with phosphate of lime, and each 100 c.c. containing 1 c.c. of 1 % solution of potassium chloride, beat almost completely for three hours, when the experiment ceased for want of time.

On the same day, a ventricle fed with the same solution, and placed in an open vessel containing the same solution, retained contractility for more than eight hours.

I find moreover that a circulating fluid composed of saline, made with tap water of the New River Company, containing to each 100 c.c., 1 c.c. of 1 % solution of potassium chloride will sustain contractility for many hours. I will now record some experiments[1] made in May and June. I fed the detached ventricle with this "tap water" circulating fluid through a perfusion cannula, the ventricle being immersed in a similar fluid.

In one experiment, ten hours afterwards, the contractions were complete on stimulation, and spontaneous beats continued a little while after its withdrawal. After twenty-seven hours the ventricle still contracted. In another experiment contractility persisted more than twenty-four hours. In another it lasted twenty-eight hours. In three others the contractility remained good for eight hours, but had disappeared next morning. In another experiment, made on June 11, the ventricle contracted completely and spontaneously 24 per minute five hours afterwards, and then quickly grew weak so that in another hour it beat very feebly. Now these experiments with phosphate of lime, also with tap water circulating fluid, unless it is maintained that the organic matter (38 parts in the million) in the tap water is adequate to nourish and sustain the heart, prove that the "ventricle can feed on itself"; in other words, it can consume and convert its own tissues into combustible material; or there is combustible substance stored up in the muscular tissue which furnishes food to support the contractions.

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[1] Except when the contrary is stated these experiments were made with Roy's tonometer and the ventricle of the English frog was employed.

We may I think infer that the arrest of contractility with a saline solution is not due to the removal of pabulum to support the contractions, but that lime and potassium salts are necessary ingredients in a circulating fluid to supply the conditions essential to the change occurring during a contraction. I am driven to the conclusion that there is most probably stored up in the muscular tissue material to carry on contractions; and that this material cannot be washed out by a fluid circulating through the cavities of the heart; and that the amount of stored-up material varies with the time of year and the condition of the frog.

Kronecker maintains that serum albumin alone is an adequate food to sustain the contractions of the frog's heart, and that peptones, white of egg, syntonin, myosin, mucin, glycogen and milk are incapable of sustaining contractility. In subsequent experiments he found that milk, boiled or unboiled, in considerable quantity can nourish the heart and sustain its contractility. So also can boiled and unboiled whey, and dialysed whey. He maintains that all measures which lessen the serum albumin in milk weaken its power to sustain contractility.

Kronecker draws attention to the great influence of even minute quantities of serum albumin in sustaining the ventricle, and my subsequent experiments amply confirm his observation.

The efficacy of a minute quantity of blood if added to a solution containing the necessary inorganic constituents is well shown in the following way. In attaching the ventricle to the perfusion cannula an incision is made through the auricles and of course the blood runs out of the ventricle; but yet enough remains clinging to the inner walls, so that, after being attached to the cannula, if 100 c.c. of "circulating fluid" is employed and is returned to the reservoir feeding the ventricle, the addition of this minute quantity of blood will sustain contractility for some minutes longer than circulating fluid alone.

On June 13, I first supplied the ventricle with 100 c.c. of circulating fluid, consisting of saline solution with 1 c.c. of calcium chloride, 1 c.c. sodium bicarbonate, and ·75 c.c. potassium chloride 1°/₀ solutions. After this 100 c.c. had been through the ventricle, carrying with it the adherent blood, I set this aside, and substituted a further quantity of a similar fluid; the contractions continued perfect for forty-two minutes and then began to grow weak. At this point I fed the ventricle with the 100 cc. containing the washings of blood mentioned, and the contractions at once improved and became complete. Then I fed the ventricle with a fresh supply of circulating fluid, and in a minute the contractions began quickly to lose in strength; then again I fed the ventricle with the 100 c.c. containing the minute quantity of blood, and in a few minutes the contractions again became perfect, but after sixteen minutes the ventricle rapidly lost strength and contractility soon ceased.

In this communication I have to record experiments, performed with the assistance of Dr Buxton, to ascertain the effect of the organized constituents of the blood and similar bodies. Like all previous observers, I of course find that blood or serum well sustains the heart's contractility; but I trust to show that blood or serum is quite inadequate to effect this unaided by inorganic substances, as lime and potassium salts, in addition to sodium chloride and an alkaline sodium salt.

It is well known that the addition of one part of whipped blood or serum to two parts of normal saline will sustain contractility for a day or more. Now I shall show that this quantity of blood or serum contains enough lime potassium and alkaline sodium salts to support contractility, and that, were inorganic salts added to simple saline, then a much smaller quantity of proteid matter would be sufficient to sustain contractility; and that without lime and potassium and alkaline sodium salts even large quantities of dialysed whipped blood or serum are inadequate to sustain normal contractions, or probably any contractions at all.

For instances I record experiments, where 1 c.c. of whipped blood, or 1 c.c. of serum, or 1 c.c. of milk added to 100 c.c. of normal saline containing physiological quantities of lime, potassium and alkaline sodium salts, sustained contractility from twenty-four to thirty-six hours. I put on record also experiments made with dialysed blood to show that blood from which the salts have been removed cannot sustain contractility; and that to sustain contractility we must add potassium and alkaline sodium salts as well as sodium chloride; and I shall further show that it is not necessary to add a lime salt, since this cannot be dialysed from blood, for after dialysis on incinerating the blood plenty of lime can be detected in the ash.

Unlike Kronecker I find white of egg albumin and gelatin capable of sustaining the heart's contractility, though both are for this purpose less efficient than blood.

In previous communications it was shown that a solution containing simply inorganic salts, namely saline solution ·6 $^0/_0$ containing 1 part in 10,000 of calcium chloride, 1 part in 10,000 of sodium bicarbonate and 1 part in 15,000 of potassium chloride will, for several hours, sustain the heart's contractility unimpaired. I find however that in certain months, notably in March and April, May and June, this solution is much less efficient.

In May and June however this circulating fluid is more effectual, if an additional quantity of calcium chloride solution is added, as for instance, ·5 c.c. of 1 $^0/_0$ solution to each 100 c.c.

During March and April, in from fifteen to thirty minutes, after the ventricle has been supplied with the fluid just mentioned (which throughout this paper will be called circulating fluid) there occurs some delay in dilatation, especially at its termination. The summit of the trace often becomes rounded. If the ventricle beats frequently, fusion occurs, with slight tonic contraction, so that the trace rises above the base line. After a short time, the contractions grow weaker till contractility ceases.

In warmer weather, as in May and June, the changes are somewhat modified. After a period varying from half an hour to an hour the summit of the trace becomes sharper, dilatation is a little delayed especially at its termination and the height of the trace becomes reduced. At this time the height of the trace depends on the frequency of the contractions. If a contraction is excited immediately on the conclusion or before the complete dilatation of the preceding contraction, the trace of the second rises less high than the trace of the first contraction. The contractions continue to grow weaker, and the effect of the length of the diastole becomes still more marked, so that the second of two contractions, one immediately following the other, may be very weak and not even a quarter the height of the preceding beat. With some hearts, when getting weak and not beating spontaneously, an excitation induces a contraction followed by a quick succession of spontaneous beats, regular or irregular in rhythm, the first beat being much stronger than the succeeding frequent contractions, and if the ventricle beats irregularly the strength of each beat depends on the length of the preceding diastole. At last the contractions grow very weak and contractility disappears.

The changes which occur in March and April are just like the changes that take place when the ventricle is fed with saline solution containing a physiological quantity of a lime salt; and a physiological quantity of a potassium salt will quite obviate the occurrence of these effects proceeding from a saline containing a lime salt. It removes the tonic contraction, accelerates dilatation, and so obviates fusion and prevents weakening of the contractions.

I will here draw attention to the fact that the changes developed during May and June are just like the effects induced by veratria.

If a small quantity of fresh blood (1 c.c. of whipped blood) or ·2 grammes of dried bullock's blood is added to 100 c.c. of circulating fluid these effects speedily disappear. The contractions strengthen again, the dilatation becomes rapid, fusion of the beats disappears, tonic contraction ceases and good contractions persist for hours.

It occurred to me that possibly the effect of blood might be due to the salts it contains. So I next experimented to ascertain whether these effects were due to the inorganic or the organic constituents of blood.

The character of the trace under the influence of circulating fluid suggested that lime effects were in excess of potassium effects. I therefore added more potassium chloride, though without effecting any change in the character of the trace; and even with the addition of large doses of potassium chloride the trace presented the same characters, but grew more rapidly weak.

The addition of a further quantity of calcium chloride with potassium chloride did not modify the character of the trace.

It is obvious then that the effects of circulating fluid in March and April are not due to the too little potassium or calcium salts contained in the fluid.

I next tested whether the addition of phosphate of sodium would influence these characters of the trace. On adding to 100 c.c. of the circulating fluid 1 c.c. of 1°/₀ solution of rhombic phosphate of sodium I found that this salt entirely prevented the delay in dilatation and for a short time strengthened the beat, but the contractions soon grew much weaker, although I added successive doses of sodium phosphate, 7 c.c. in all. Sodium phosphate therefore favours dilatation and so prevents the delay which occurs with circulating fluid, but it does not, like small quantities of blood, strengthen and sustain the ventricular contractions.

I next experimented to ascertain whether this action of phosphate of sodium could be due to its alkalinity. I added sodium bicarbonate to the circulating fluid and find that this acts just like sodium phosphate, accelerating dilatation without sustaining the contractility of the ventricle.

It is obvious therefore that the effects of a small quantity of blood added to the circulating fluid cannot all be attributable to the inorganic constituents, but are in all probability due to the organic substances.

In order to prove this more conclusively, I dialysed both dried blood and fresh bullock's blood to separate all the salts and then tested the effect of the dialysed blood.

I made a series of experiments with dialysed dried blood and a similar series with dialysed fresh blood. I dialysed the blood from two to seven days, and during the whole of this time it remained quite fresh. Prolonging the dialysis beyond two days does not further modify the effect of the dialysed blood.

With one exception the dialysed dried blood behaved in all respects like dialysed fresh blood.

This dialysed blood (1 c.c. to 100 c.c.) quite removes or prevents the effects of circulating fluid set forth at the beginning of this communication and maintains perfect contractions for many hours.

It was necessary now to make experiments to ascertain if the salts were separated by dialysis from the proteid substances of the blood.

I mixed dialysed dried blood in distilled water so that the solution contained the equivalent of 50°/₀ of fresh blood. Having taken a trace with 100 c.c. of circulating fluid, containing ·2 grammes of dried blood, I replaced this by 100 c.c. of the solution of dialysed blood. The ventricle quickly passed into systole, just as the ventricle does when fed with distilled water alone, an effect prevented entirely by the addition of sodium chloride to distilled water.

This experiment plainly shows that the greater part if not the whole of the sodium chloride is dialysed out.

On adding ·6 grammes of sodium chloride to the dialysed blood solution the persistent spasm speedily relaxed, though only weak contractions ensued. I added first 1 c.c. of 1°/₀ solution of calcium chloride, which quickly strengthened the contractions, and next 1 c.c. of 1°/₀ sodium bicarbonate solution. Perfectly good contractions returned, but they showed the character of a trace recorded by a ventricle fed with saline solution containing calcium chloride and sodium bicarbonate, unantagonised by a potassium salt.

So I then added ·75 c.c. of 1°/₀ solution of potassium chloride, and this dose quite obviated the delay in dilatation, and produced normal contractions just like those at the beginning of the experiment.

The delay in dilatation and the effect of a physiological dose of potassium chloride (1 in 15,000) in preventing this delay show that all or most of the potash salts were dialysed out of the organic constituents.

The same occurs with dialysed fresh blood.

These experiments I then repeated in a somewhat modified form.

I took a trace with 100 c.c. circulating fluid containing ·2 grms. of dried blood and replaced this by 4 grms. of dialysed dried bullock's blood in 100 c.c. distilled water, and added 1 c.c. of 1°/₀ solution of calcium chloride and 1 c.c. of 1°/₀ solution of sodium bicarbonate. This solution threw the ventricle into a state of persistent spasm (tonic contraction). Then I added ·6 grms. of sodium chloride which relaxed the spasm with return of good contractions with very great delay in dilatation. The addition of ·75 c.c. of 1°/₀ solution of potassium chloride removed the delay in dilatation and produced quite normal beats as good almost as at the beginning of the experiment. These effects are shown in Plate IX. Figs. *A*—*G*.

This experiment shows moreover that dialysis removes the whole or the greater part of the potassium salts from the blood.

I then made the following series of experiments:

I took a trace of the ventricle fed with circulating fluid, and replaced it with 50 c.c. of fresh bullock's blood (dialysed for five days), with 50 c.c. of distilled water, containing ·6 grms. of sodium chloride. Contractility remained good, but the character of the trace became modified, and assumed the characters that occur when the ventricle is fed with saline solution containing a physiological quantity of a lime salt. The strength of the contraction increased so that the trace rose higher. The trace became broader also and rounded at its summit, and then dilatation became delayed, causing fusion of the beats and some tonic contraction. The subsequent addition ·75 c.c. of 1°/₀ solution of potassium chloride (1 to 15,000) obviated most of these effects. The dilatation became accelerated, the fusion and contraction ceased, and a normal trace ensued similar to the trace at the beginning of the experiment. The contractions soon grew weak, but again became quite strong on adding 1 c.c. of $1^{\circ}/_{0}$ solution of calcium chloride. These effects are well shown in Plate IX. Figs. *H—O*.

In repeating this experiment, but with blood dialysed for eight days, I obtained the same results.

The same result followed with dialysed dried bullock's blood, but with this difference :—that after adding potassium chloride, to obviate the delay in dilatation, the heart soon began to beat very incoordinately and the addition of calcium chloride and sodium bicarbonate rather increased than lessened this irregular action.

In this experiment then, after five (and in another experiment eight) days of dialysis, on adding ·6 grms. of sodium chloride to a mixture composed of 50 c.c. of blood and 50 c.c. of distilled water, the contractility remained good, but the contractions assumed the character they display when the ventricle is supplied with saline containing only a calcium salt. A physiological quantity of potassium chloride obviated these effects, but after the addition of this the contractions, though normal in character, grew feeble. From these experiments it is obvious either that the nitrogenous constituents are capable of sustaining contractility without the intervention of lime, and that they produce the same character of trace as is produced by lime, and that these effects are obviated, as with lime salts, by a minute quantity of potassium chloride, or lime salts are combined with the organic constituents of the blood and cannot be dialysed out, and thus affect the ventricle, causing the same character of trace which follows the addition of lime salts to saline solution.

I next tested whether dialysed blood contains lime. I dialysed dried bullock's blood for three days, first dissolving it in saline. I then evaporated and incinerated it, and tested the ash by dissolving some of it in weak hydrochloric acid, and neutralizing the filtrate with ammonia. On adding ammonium oxalate an abundant deposit of calcium oxalate occurred. On another occasion I dialysed the blood for eight days with the same result. The lime therefore is not all removed by dialysis, and probably the delay in dilatation we have just discussed is due to the retained lime combined with the organic constituents.

Some lime we see is retained after dialysis, and this at least assists to maintain the contractility; but even if all the contractility sustained by dialysed blood is due to lime still only a small quantity of it is retained, for the addition of a physiological quantity of potassium chloride greatly weakens the contraction.

I must conclude then that dialysis removes the sodium and potassium salts but is unable to remove all the calcium salt; enough remaining to cause good contractions when no potassium salts are present, but sufficient only to sustain weak contractions when a physiological quantity of a potassium salt is present.

I have shown then that inorganic salts even with blood are necessary to the contraction of the ventricle. This is certainly the case with sodium chloride, potassium chloride, and an alkaline salt. Even were it proved that serum albumin has some of the properties of a calcium salt, and with the aid of sodium chloride can sustain contractility, still I have shown that serum albumin is insufficient in the presence of a potassium salt to sustain good contractions, and that to sustain full contractions a calcium salt must be added.

I shall next show that when the circulating fluid contains sufficient quantity of certain inorganic salts a very small quantity of blood, fresh or dried, or serum, or milk, is sufficient to sustain contractility for many hours, even at seasons when circulating fluid by itself will sustain contractility only half an hour to an hour. Thus, the addition of 1 c.c. of whipped blood to the 100 c.c. of circulating fluid will maintain contractility more than twenty-four hours. The addition of ·2 to ·4 grammes of dried blood to the 100 c.c. will sustain perfect contractility from three to four hours; probably even much longer, but I have not continued the experiment beyond this time. Again, 1 c.c. or 2 c.c., or better, 3 c.c. of milk added to the 100 c.c. of circulating fluid will sustain contractility more than twenty-four hours.

With these small quantities of organic materials however it is necessary to have sodium, and potassium, and calcium salts in definite proportion, for if these organic fluids are added to simple saline they prove quite inadequate to support contractility. However, on adding the necessary quantity of potassium, calcium and sodium bicarbonate salts contractility at once returns and becomes complete.

If the blood and serum albumin act as food to the ventricle, as Kronecker maintains, it must be the proteid substances that feed the muscle and not the carbo-hydrates or fats. For Kronecker finds glycogen incapable of sustaining contractility, and in some of the experiments he records, the dialysed blood sustained contractility effectively, and probably the carbo-hydrates would be separated by dialysis.

I washed dried blood carefully with ether, and on April 28, the temperature of the room being 18° C., I first fed the ventricle with circulating fluid. In an hour the height of the trace was reduced to about one half. I then fed the ventricle with 200 c.c. of circulating fluid containing ·4 grammes of dried ether-washed blood. In eight minutes the ventricle had quite recovered and contracted completely; and fifty minutes afterwards the ventricle still beat well, the contractions being only a very little weakened.

I conclude then that the effect of blood added to circulating fluid is not dependent on the fats it contains.

If we accept the view generally held, that blood acts as food to the heart and so sustains contractility, the question then arises, which of the nitrogenous organic constituents possesses this sustaining power, the serum albumin, the globulin, hæmoglobin or peptones? Moreover, can nitrogenous bodies other than those present in the blood sustain contractility. Kronecker, Martius and Ott maintain that serum albumin is the only proteid that can sustain the heart, and they find peptones, casein, white of egg, syntonin, myosin, mucus, and glycogen incapable of sustaining contractility.

I first tried white of egg. I added distilled water to white of egg so that the solution contained 40 parts of white of egg in 100 parts of the mixture, and carefully filtered it. I added 2 c.c. of this mixture to the 100 c.c. of circulating fluid and performed many experiments varied in different ways.

On April 2, the temperature of the room being 14° C., I supplied the ventricle with circulating fluid containing white of egg mixture. Ninety minutes afterwards the ventricle still contracted perfectly. Then I replaced the circulating fluid and white of egg by 100 c.c. of circulating fluid, to which were added 1 c.c. of 1 °/₀ solution of calcium chloride and 1 c.c. of 1 °/₀ solution of sodium bicarbonate. These salts were added because it seemed possible that the effect of white of egg might be due to its salts, and not to its proteid substances. The contractions soon grew weaker, and dilatation became delayed. When the height of the trace was depressed about one half I again fed the ventricle with circulating fluid containing white of egg; the contractions at once increased in strength without becoming quite so strong as they were previous to the use of simple circulating fluid. Forty minutes later, and about 160 minutes from the beginning of the experiment, the contractions had become a little weaker. This experiment clearly shows that white of egg will greatly aid the inorganic constituents of circulating fluid to sustain contractility.

I find that 1 c.c. of 40 parts of white of egg in 100 quite sufficient to greatly enhance the efficacy of 100 c.c. of circulating fluid to sustain contractility. If however this quantity of white of egg is added to 100 c.c. of saline solution the contractions rapidly grow weaker and contractility soon ceases, but at once returns on the addition of 1 c.c. of 1 °/₀ solution of calcium chloride; and the contractions become quite normal on the further addition of ·75 c.c. of 1 °/₀ solution of potassium chloride. This quantity of white of egg without lime and potassium chloride is therefore unable to sustain contractility.

Like dialysed whipped blood so dialysed white of egg, when added to saline solution, supports contractility but alters the trace just like lime; the dilitation becomes much delayed, and if the contractions are of normal frequency then fusion of the beats occurs. A small dose of potassium chloride will completely prevent the occurrence of these.

As on incineration the ash yields evidence of plenty of lime, I conclude that these results are due to the lime combined with the proteid substance which cannot be dialysed out.

Like Kronecker I find that milk very effectively sustains the heart's contractility; but unlike him I find that a very small quantity, 2 c.c. to 100 c.c., is sufficient if to the circulating saline we add, besides the milk, alkaline sodium, potassium and calcium salts.

On April 22, the temperature of the room being 23° C., I first fed the ventricle with circulating fluid. In about fourteen minutes, after the ventricle had grown much weaker, I added to the 100 c.c. of circulating fluid 1 c.c. of milk; a great improvement in the contractions resulted; and on adding another cubic centimetre, the contractions became as good as at the beginning of the experiment. Again, I fed the ventricle with circulating fluid until the contractions were reduced to one half their previous strength; yet I again added the same quantity of milk, and perfect contractions returned. Once more on feeding the ventricle with circulating fluid, and when it had grown so very weak as scarcely to contract at all, I added milk and again improved the beat, though only to a little more than half the strength of the beat at the commencement of the experiment.

I subsequently fed the ventricle with circulating fluid containing some dissolved dried blood, but without increasing the strength of the beat.

On April 23, the temperature of the room being 19° C., I repeated this experiment with milk.

I first took a trace with 100 c.c. circulating fluid, and in about twenty-seven minutes the beats were only half as complete as at the beginning, and rapidly grew weaker; experience showing that in a few minutes more contractility would have well nigh ceased. I then added 2 c.c. of fresh unskimmed milk. The beats slowly improved, and in about four minutes had quite recovered, and one hour and forty-five minutes afterwards the ventricle still beat perfectly; the contractions being just as strong as at the beginning of the experiment.

I next performed on May 9 three experiments, using in the first 1 c.c. of milk to 100 c.c. of circulating fluid, in the second 2 c.c. to 100 c.c. of circulating fluid, and in the third 3 c.c. to 100 c.c. of circulating fluid. In each experiment the fluid flowed through the ventricle by means of a perfusion cannula, and the ventricle was immersed in a solution of the same composition as that circulating through it.

In the first experiment the ventricle contracted fully and spontaneously 10 hours, but next morning contractility had ceased; in the second experiment the ventricle beat feebly on excitation after twenty-four hours; and in experiment three the ventricle still spontaneously contracted about one half its original force twenty-four hours from the beginning of the experiment.

Milk then like blood effectively sustains the contractility of the ventricle. If added to saline only, then larger quantities are necessary, so that the milk may contain sufficient of the necessary inorganic salts. But if these inorganic salts are added to the saline solution then 2 to 3 c.c. to the 100 c.c. of fluid will sustain contractility more than twenty-four hours. It is well to state that the frogs were weak, having been kept a long time in the tanks.

I next tested the action of gelatin. I employed Swinbourne and Nelson's carefully prepared gelatin. On April 24, the temperature of the room being 16° C., I took a trace with circulating fluid. In about forty-eight minutes, on the ventricular contractions beginning to grow weak, I added ·5 gramme of gelatin to the 100 c.c. of circulating fluid. The contractions completely recovered their lost strength, and so continued forty-eight minutes after adding the gelatin. I then replaced the above mixture by 100 c.c. of saline solution with ·5 gramme of gelatin. The contractions quickly grew very weak. I then returned to the circulating fluid containing gelatin, and the contractions improved till they became as good as at the beginning and so remained for twenty-four minutes, when I was obliged to discontinue the experiment.

This experiment I repeated with the following result. On April 24 I first took a trace with circulating fluid. In about fifty-six minutes the trace fell to nearly one half its original height. I then added ·25 gramme of gelatin to 200 c.c. of circulating fluid and the contractions slowly recovered the greater part of their lost strength. I added then more gelatin, a gramme in all to the 200 c.c. of circulating fluid, and quite restored the beats. I then supplied the ventricle with 100 c.c. of circulating fluid containing ·25 gramme of gelatin; the contractions continued strong, and two hours from the commencement of the experiment the ventricle still beat perfectly.

Numerous experiments show that ·25 gramme of gelatin added to 100 c.c. of circulating fluid will maintain perfect contractions for several hours.

I then made on May 6 a series of three experiments. In the first I employed a gelatin solution ·5 gramme of Swinbourne's best gelatin in 100 c.c. of circulating fluid. Ten hours later the ventricle on stimulation beat fairly well.

In the second experiment I added to each 100 c.c. of circulating fluid 1 c.c. of blood preserved by adding thymol to it. For seven hours the ventricle beat spontaneously. Ten hours from the beginning the ventricle contracted nearly completely on stimulation, and twenty-four hours later the ventricle still beat feebly.

In the third experiment I fed the ventricle with circulating fluid. In forty minutes the beats were very feeble, indeed the ventricle nearly stopped. I then added 1 c.c. of thymolized[1] blood to the 100 c.c. of circulating fluid and the contractions speedily improved, five hours after they remained fair, and in twenty-four hours the ventricle, on stimulation, still contracted somewhat better than No. II.

On May 7, I repeated this series. The ventricle fed with gelatin solution lost its contractility in about six hours. The ventricle fed with thymolized blood solution ceased contracting in seven hours. The ventricle fed with circulating fluid had in fifteen minutes almost lost its contractility; but, on adding thymolized blood to the circulating fluid, the contractions greatly improved, the ventricle contracting about one-third, and continued for eight hours from the beginning when the experiment was discontinued.

In these experiments the ventricle was fed through a perfusion cannula, and the heart was immersed in a solution of the same composition as that circulating through it.

On testing Swinbourne's commercial gelatin with ferrocyanide of potassium and acetic acid I found that it contained a considerable quantity of proteid matter. This was separated from the gelatin by soaking it in distilled water for three days, changing the water daily. The gelatin was then dissolved by heat and poured into alcohol, and the precipitate tested as above showed the gelatin to be pure or to contain at most only an infinitesimal quantity of proteid matter.

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[1] Whipped blood preserved by thymol.

I first fed a ventricle with circulating fluid and in a little over an hour the contraction became very weak, when I added about ·5 gramme of pure gelatin to the 100 c.c. of circulating fluid. The contractions greatly improved in strength, and so remained fifty minutes after the addition of gelatin, though the contractions were not so strong as at the beginning. On supplying the ventricle with circulating fluid containing thymolized blood (1 c.c. to 100 c.c.) the contractions became complete.

On May 15 I fed a ventricle with circulating fluid containing pure gelatin, about ·5 grm. to 100 c.c. of fluid. Ninety-six minutes from the beginning the ventricle beat as strongly as at first. It then gradually grew weaker. Three hours from the start, the trace of the contraction had diminished to about one-third the height of the trace at the beginning of the experiment.

On May 13 I performed two experiments. I fed one ventricle with a solution containing about ·5 grm. of gelatin in 100 c.c. of circulating fluid, and in second experiment I fed another ventricle with a solution containing double this quantity of gelatin.

In the first experiment the ventricle beat feebly on stimulation about seven hours later. In the second experiment the ventricle beat only about four hours.

Next day I repeated the experiments using the same solutions.

In No. I. the ventricle retained its contractility for six hours and a half. After the ventricle lost its contractility I replaced the gelatin solution by 100 c.c. of circulating fluid containing 1 c.c. of thymolized blood, and the ventricle at once commenced contracting. In No. II. the contractility ceased in five hours. I then replaced the gelatin fluid by circulating fluid containing blood (thymolized) 1 c.c. to 100, and the contractility returned and persisted twenty-four hours from the beginning of the experiment.

A test heart fed with circulating fluid quite lost its contractility in an hour and twenty-five minutes.

On May 15 I repeated this experiment, using the same solutions.

No. I. lost its contractility in eight hours. No. II. also in eight hours.

Gelatin then like blood and milk helps greatly to sustain contractility, but in this respect is decidedly inferior to blood.

DESCRIPTION OF PLATE IX.

A—G. Experiment with dialysed dried bullock's blood.

A shows the character of the contractions with the ventricle supplied with 100 c.c. circulating fluid containing ·2 gramme of dried bullock's blood.

B shows the effect of replacing this by 100 c.c. distilled water containing 1 c.c. of $1^0/_0$ solution of calcium chloride, and 1 c.c. of $1^0/_0$ solution of sodium bicarbonate, and 4 grammes of dried blood dialysed for two days.

C shows the effect of adding ·6 gramme of sodium chloride.

D shows the effect eight minutes later.

E shows the effect twenty-two minutes after the replacement.

F shows the effect of adding to the fluid ·75 c.c. of $1^0/_0$ solution of potassium chloride.

G shows the trace sixteen minutes after adding the potassium chloride.

H—O. Experiment 50 c.c. whipped blood dialysed for five days with 50 c.c. of distilled water containing ·6 gramme of sodium chloride. March 20. Temp. of room 13·5 C.

H. Trace taken with circulating fluid.

I. The effect of replacing this with the dialysed blood solution eight minutes after the substitution.

K shows the trace sixty-two minutes after the replacement.

L shows the effect of adding ·75 c.c. of $1^0/_0$ solution of potassium chloride.

M. The trace eight minutes later.

N. The effect of adding 1 c.c. of $1^0/_0$ solution of calcium chloride. Subsequently added 1 c.c. of $1^0/_0$ solution of sodium bicarbonate.

O shows the trace three hours from the commencement of the experiment.

PART II.

I shall now attempt to ascertain why a circulating fluid made with tap water sustains contractility for hours at a time of year when "circulating fluid"[1] made with distilled water will only sustain good contractions for half an hour to three-quarters of an hour. I find that a circulating fluid composed of tap water saline containing one part of potassium chloride in 10,000 parts, when supplied to the ventricle through a perfusion cannula and the ventricle is immersed in a similar solution, will sustain contractility for more than twenty-four hours. Again, the same solution sustained perfect contractions in a ventricle employed in

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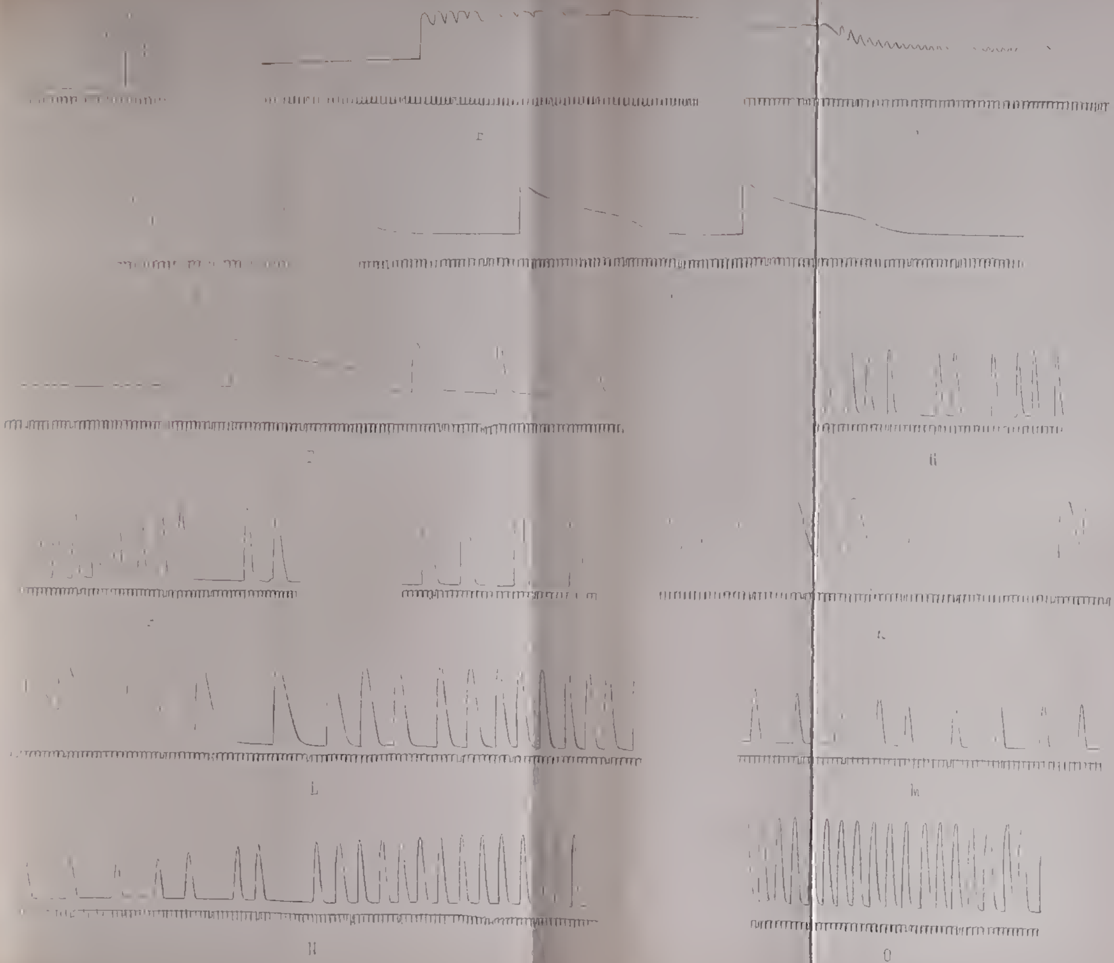
[1] As I have already stated "circulating fluid" consists of 100 c.c. saline $·6^0/_0$ with 1 c.c. of $NaHCl^3$, 1 c.c. $CaCl^2$ and ·75 c.c. of KCl $1^0/_0$ solutions.



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Roy's apparatus for three hours, when for lack of time I discontinued the experiment.

Now New River water contains 38 parts per million of organic matter. Does this minute quantity of organic matter feed the ventricle and sustain contractility[1] or does tap water contain inorganic constituents adequate to sustain the contractility so completely and for so long a time?

I boiled tap .water for half an hour, thinking that this would probably coagulate the organic matter in the water. After filtering the fluid to separate the precipitated bicarbonate of lime I added to each 100 c.c. ·6 grammes of sodium chloride, 1 c.c. of calcium chloride, 1 c.c. of sodium bicarbonate and ·75 c.c. of potassium chloride 1°/₀ solutions. Two hours and a half afterwards the contractions were as good as at the beginning of the experiment.

On June 17 I boiled tap water for four hours, filtered and made it into ·6°/₀ saline, and added to each 100 c.c. 1 c.c. of 1°/₀ solution of potassium chloride. This fluid sustained only weak contractions, which became much stronger on adding 1 c.c. of 1°/₀ solution of calcium chloride, 1 c.c. of sodium bicarbonate 1°/₀ solution to each 100 c.c., and good contractions continued for an hour. Restoration too was rapid and considerable, for a contraction excited before the preceding dilatation was completed was more complete than the preceding contraction. In about seventy minutes some persistent spasm set in, which lessened but did not disappear on adding ·5 c.c. of 1°/₀ solution of potassium chloride.

On the same day I experimented with tap circulating fluid. At the end of an hour and fifteen minutes the contractions were nearly as good as at the beginning, but reparation was not normal; the second of two contractions occurring close together being weaker than the first contraction.

The rapid restoration then is not due to the ingredients of tap water, but to some change induced by boiling, and is probably due to suspended carbonate.

The following experiments confirm this surmise:

The same day I boiled tap water for six hours and allowed it to stand till next morning, and then drew off the upper part of the fluid by a syphon and filtered it through three pieces of filter paper. I then made it into ·6% saline and added to each 100 c.c. 1 c.c. of calcium chloride, 1 c.c. of sodium bicarbonate and 1 c.c. of potassium chloride 1% solutions. For an hour and thirty-five minutes the contractions remained complete. The height of the trace then very slowly declined. In two hours and ten minutes from the beginning the trace was about a sixth less high than at the beginning of the experiment. During the latter half of this time reparation was delayed so that in exciting two contractions one immediately after the other, or one before dilatation had completed, the second contraction was weaker than the preceding one. I then added to the circulating fluid some of the insoluble deposit thrown down on boiling tap water, i.e. carbonate of lime, and immediately reparation became greatly accelerated, so that the second of two contractions, occurring closely together, was greater than the first; and if a contraction was excited, some time even before the preceding dilatation was completed, the trace was higher than the first contraction and there occurred "piling up." This occurred in the preceding experiment, and so no doubt some carbonate of lime passed through the filter and affected the ventricle, but the quantity must have been very small as the filtered fluid was quite clear.

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[1] Subsequent to these experiments I found that a circulating fluid composed of a saturated solution of phosphate of lime in ·6°/₀ saline, containing to each 100 c.c. ·5 c.c. 1°/₀ solution of KCl, will sustain contractility for many hours, and is indeed almost equal to tap water circulating fluid.

I then evaporated down to dryness about 50 c.c. of this boiled and filtered water and incinerated, and the slight residue burned brown and then black; and on further heating it the colour quite disappeared, showing that the water contains a small trace of organic matter. I find that distilled water also always contains a trace of organic matter, though less than boiled tap water.

This experiment then has not enabled us to determine whether the efficacy of tap water is due to its inorganic constituents.

I next tested how far the addition of a minute quantity of organic matter to circulating fluid would enable it to sustain contractility equal to a circulating fluid made with tap water.

As the organic matter of blood must, one would imagine, be more efficacious than the organic matter of tap water I mixed 1 gramme of dried bullock's blood with 100 c.c. of distilled water, and found that about ·8 gramme were so dissolved that the solution passed through Swedish filtering paper; I then added various quantities of this ·8% solution to circulating fluid.

On June 19 I fed the ventricle with circulating fluid containing to each 100 c.c. ·5 c.c. of a ·8% solution of dried blood. The contractions remained perfect for thirty-five minutes and then dilatation became delayed, and in a few more minutes the height of the trace became reduced, and dilatation became still more delayed. I then replaced the fluid by tap circulating fluid and the contractions at once recovered, but the trace became broader than at the beginning of the experiment.

This experiment shows that one part of dried blood in about 24,000 parts of circulating fluid cannot sustain contractility as well as tap water saline.

On the same day, June 19, I fed a ventricle with circulating fluid. In about twenty minutes the dilatation began to be delayed, and in twenty-five minutes the contractions occurred in groups whose beats fused, and in thirty minutes the contractions rapidly grew weaker and all but ceased, when I added 1 c.c. of ·8°/$_0$ solution of dried blood to 100 c.c. of circulating fluid but without improving the contractions. Forty minutes from the commencement I substituted tap water circulating fluid, and at once the contractions rapidly improved and became nearly as complete as at the beginning of the experiment, but dilatation was much delayed. I then fed the ventricle with 100 c.c. circulating fluid containing 1 c.c. of ·8°/$_0$ solution of dried blood, and the contractions again rapidly grew very weak, and again improved on feeding the ventricle with tap water circulating fluid, but did not recover their original strength.

On June 5 I fed the ventricle with circulating fluid. In twelve minutes the spontaneous contractions grouped themselves in phases and fused a good deal (much as happens with a small quantity of veratria). The height of the trace then quickly declined, and in twenty minutes it was not a quarter so high as at the commencement. I then added 1 c.c. of dissolved blood to 100 c.c. of circulating fluid, but without improving the contraction; nor did the subsequent addition of 1 c.c. of calcium chloride nor 1 c.c. of sodium bicarbonate 1°/$_0$ solutions effect any improvement.

On another occasion I first fed the ventricle with circulating fluid to which I added ·5 c.c. of 1°/$_0$ solution of calcium chloride to each 100 c.c. After forty minutes the contraction began to grow weak, and when the height of the trace was reduced about a sixth I added to each 100 c.c. of fluid 1 c.c. of ·8°/$_0$ solution of dried blood. The contractions completely recovered, but again began to grow weak in about twelve minutes, and in another four minutes the height of the trace was reduced to about a third its original height. I then replaced the circulating fluid containing blood with tap water circulating fluid, composed of 100 tap water saline containing 1 c.c. of 1°/$_0$ solution of potassium chloride. The contractions speedily became complete. In about eighty-five minutes the contractions again began to grow weak, and two hours after the use of tap water circulating fluid the height of the trace was reduced by one half.

These experiments prove that circulating fluid containing 1 part of dried blood in about 12,000 is decidedly inferior to tap water circulating fluid which contains one part of organic matter in about 25,000, and they therefore prove that the efficacy of tap water saline is not entirely, if it is at all, dependent on the organic matter it contains.

These experiments and others that I will now add show the great effect of a minute quantity of organic matter, for one part of dried blood in about 12,000 of circulating fluid will sustain good contractions for many hours.

On June 8 I added 1 c.c. of ·8% solution of dried blood to each 100 c.c. of circulating fluid, and two hours afterwards the contractions were as good as at the beginning of the experiment.

On June 9 I repeated this experiment. In two hours and three-quarters the height of the trace was only a tenth less high than at the beginning of the experiment.

On June 15 I repeated this experiment. The contractions remained quite good for an hour and a half, and then grew a little weaker. On adding another cubic centimetre to the 100, i.e. one part of blood in 6,000, the contractions completely recovered. In three hours the height of the trace was a shade less than at the start.

We see then that whilst circulating fluid containing one part in 12,000, will long sustain contractility, yet it is decidedly inferior to tap water circulating fluid containing only one part of organic matter in 25,000.

As then the efficacy of tap water circulating fluid cannot be due, or only in a small part due, to its organic matter I next tested whether its lime salts, namely bicarbonate of lime and sulphate of lime, are more efficacious than chloride of calcium.

I made a calcium bicarbonate solution by diluting lime water with an equal quantity of distilled water and poured carbonic acid rapidly through it. The greater part of the carbonate precipitate was redissolved as bicarbonate, though some turbidity remained which settled, and I obtained thus a clear fluid.

In making a circulating fluid I added 100 of this bicarbonate of lime solution to 200 distilled water and 1·8 grammes of sodium chloride and 2·25 c.c. of 1% solution of potassium chloride. The contractions continued good for an hour and twenty minutes. Then dilatation became a little delayed. The contractions grouped in phases and fused and grew weak, and ceased in about two hours and twenty minutes.

On June 3 I repeated this experiment, but added 3 c.c. 1% solution of potassium chloride to the 300 c.c. of fluid. In thirty minutes the contractions became decidedly weaker.

On the same day I made a control experiment with tap water circulating fluid made of tap water saline with 1 c.c. of 1% solution of potassium chloride to the 100 c.c. of saline. Two hours and three-quarters afterwards the ventricle contracted as completely as at the beginning of the experiment, but dilatation was a little delayed.

It appears then that although bicarbonate of lime can sustain contractility longer than chloride of calcium, still it is far less efficacious than tap water, and consequently the efficacy of tap water circulating fluid is probably not chiefly due to its bicarbonate of lime.

I next made a saturated solution of pure sulphate of lime (obtained of Hopkins and Williams) in distilled water.

This I made into saline solution of the strength ·6%.

On June 3 I fed the ventricle with 200 c.c. of sulphate of lime saline solution, with 200 c.c. saline solution, with 4 c.c. of sodium bicarbonate and 3 c.c. of potassium chloride 1% solutions. As lime salt was decidedly in excess of the potassium salt I added in successive doses more potassium chloride solutions up to 6·5 c.c. in all. In eighty minutes on the completion of the experiment the ventricle contracted perhaps a little less perfectly than at the beginning, and reparation was rapid for, no matter how frequent the contractions, they were equally complete.

On June 4 I repeated this experiment, using however only 100 c.c. of sulphate of lime saline to 300 c.c. of simple saline and added 4 c.c. of sodium bicarbonate and 6 c.c. of potassium chloride 1% solutions. This quantity of potassium chloride proving insufficient I added in successive doses another 6 c.c. In an hour and ten minutes from the beginning of the experiment the contractions were about an eighth weaker, but reparation was rapid.

I repeated the experiment on the same day. In an hour and thirty minutes the contractions were only a third of their completeness at the beginning of the experiment.

Sulphate of lime then is decidedly more efficacious than chloride of calcium, and much of the efficiency of tap water is probably due to its lime sulphate but not entirely, so I next tested the effect of a combination of calcium sulphate and calcium bicarbonate; but although I have combined them in variable proportions I have failed to concoct a circulating fluid equal to that made with tap water.

[*From the Journal of Physiology. Vol. VII. No. 2.*]

A FURTHER CONTRIBUTION REGARDING THE EFFECT OF MINUTE QUANTITIES OF INORGANIC SALTS ON ORGANISED STRUCTURES.

By SYDNEY RINGER, M.D., F.R.S., *Professor of Medicine at University College, London.*

THE effect of minute doses of lime and other salts in maintaining the heart's contractility led me to test the action on fish of distilled water alone and of distilled water holding inorganic salts in solution. Fish I found soon died when placed in distilled water, a result due not to the absence of air but to the absence of salts in the water. Distilled water kills in one of two ways or by both combined. It may cause the diffusion from the blood through the gills of lime and other salts necessary for the maintenance of muscular contractility; or it may act through diffusion and osmosis or imbibition on the tissues of the gills, disorganizing them and so arresting respiration.

To ascertain in what manner distilled water destroys fish, I tested the action on the gill edges of fresh water mussels of distilled water alone and of distilled water containing various proportions of salts. I cut off the edges of the gills, and placed a certain portion in the several solutions and in tap water, and examined them daily with the microscope. Distilled water I found soon destroyed ciliary action, causing the cells to swell often to quadruple their natural size, so as to burst many and liberate their nuclei. The distilled water also separated the cells, causing in fact complete disintegration of the tissue. Distilled water not only causes the cells to swell and burst, but separates them from one another, and appears to induce swelling of the cement material which unites the cells. It is evident therefore that distilled water, in part at least, will destroy life by disorganizing the gills and impeding respiration.

This separation and disintegration of the structure must be caused either by osmosis and diffusion or by imbibition.

In this paper I record observations made, with the assistance of Dr Buxton, to still further learn the effect of minute quantities of saline ingredients on organized structures.

It is well known that Laminaria, a fucoid alga of cellular structure, without woody or vascular tissue, when dried and immersed in water, swells enormously; and it occurred to me that, if inorganic salts would modify the swelling of this vegetable structure, this experiment might throw some light on the mode of action of lime on animal structures.

In the fucoid alga the cell wall consists of two parts, the outer being composed of a degradation compound, namely bassorin or tragacanth, and this structure it is which swells up so enormously when the dried plant is immersed in water. I shall proceed to show this swelling is due mainly at least to imbibition by the bassorin (algin), and further that lime salts will control to a great extent the amount of imbibition. This great swelling of laminaria must be due either to imbibition or to osmosis or to both. The osmosis however must play but a small part, for the osmotic equivalent of sodium chloride is itself small and only somewhat greater than that of calcium chloride. Osmosis therefore, it is obvious, does not account for the prodigious tumefaction of laminaria.

I tested the effect of solutions containing lime salts, sodium salts, and potassium salts respectively. I employed a 1 °/₀ solution of chloride of calcium, adding various quantities to distilled water, using in each experiment 400 c.c. of fluid.

I placed portions of laminaria in 400 c.c. of each testing fluid, which I each day renewed. In each series I placed a piece of the same stem of exactly the same weight in each solution, and each day tested the amount of imbibition by first wiping and then weighing the laminaria.

I first tested the effect of distilled water and of tap water, making many observations though I record only two.

I placed two pieces of laminaria, each weighing 2 grammes, one in distilled water, the other in tap water. On the fifth day the piece from distilled water weighed 11·9 grammes and that from tap water 6·75 grammes.

In another experiment I immersed a piece of laminaria in distilled water and another piece exactly the same weight in tap water, and on the fifth day they weighed respectively 5·8 and 3·85 grammes. Then I dried both pieces and placed them in 2 °/₀ solution of sodium chloride (which I shall subsequently show obviates the effect of lime), and their weights on the fifth day were respectively 5 and 4·7 grammes.

These pieces of laminaria I again dried. Reversing the experiment, I immersed the one formerly in distilled water in tap water, and vice versa. In eight days the tap water laminaria weighed 5·35 grammes and that placed in distilled water 6·75 grammes.

These figures then show that laminaria when immersed in distilled water absorbs more fluid than when placed in tap water. Of course the difference is more marked when large pieces of dried laminaria are used.

With a piece of dried laminaria weighing 2 grammes the difference amounted at the end of five days to 5 grammes.

This difference between the effect of distilled and that of tap water on laminaria must be due to the saline constituents of tap water. The saline constituents of tap water being chiefly composed of sodium, potassium, or calcium salts, I next tested which of these salts lessens the absorbent quality of laminaria.

I immersed pieces of laminaria respectively in distilled water and in 0·5, 1, and 2 % solutions of sodium chloride. I made many series of experiments which it is not necessary to record, for they all gave the same result. The piece of laminaria in distilled water swelled more rapidly than the others in saline solutions, but after a time the pieces in saline solutions caught up the one immersed in distilled water and in many instances reached a greater weight.

I made observations also with solutions of potassium chloride of the same strength, and the results were identical with the experiments made with sodium chloride, i.e. the portions of laminaria swelled more slowly in potassium solutions than in distilled water; but in about fourteen days the laminaria in potassium solutions reached the same weight as the pieces in distilled water.

The sodium and potassium salts therefore are not the ingredients in tap water which lessen the imbibition by laminaria.

I next tested the action of calcium chloride on the imbibition by laminaria, and find that very small quantities of lime lessen considerably the amount of fluid taken up.

I took four pieces of dried laminaria each weighing 4·8 grammes. The first I placed in distilled water; the second in a solution containing 5 c.c. of calcium chloride solution to 395 c.c. of distilled water; the third in a solution containing 10 c.c. of calcium solution to 390 c.c. of distilled water; and the fourth in a solution containing 20 c.c. of calcium chloride solution to 380 c.c. of distilled water. In seven days the first weighed 22·35 grms., the second 17·25 grms., the third 14·3 grms., the fourth 11·5 grms.

In a second series of testings I repeated this experiment and added two other solutions, the fifth solution consisting of 40 c.c. of calcium chloride solution to 360 c.c. distilled water, and the sixth solution consisted of 80 c.c. calcium chloride solution to 320 c.c. distilled water.

In each solution I immersed a piece of laminaria weighing 3·65 grms., and on the seventh day the first, i.e. that in distilled water, weighed 20·6 grms., the second 14·2 grms., the third 11·5 grms., the fourth 8·6 grms., the fifth 8·15 grms., and the sixth 6·35 grms.

These experiments prove the powerful effect of calcium chloride in lessening the imbibition of fluid by laminaria. One part of calcium chloride in 9000 parts of distilled water sensibly diminishes the absorption of fluid; for instance, in one case, the laminaria in distilled water weighed 22 grammes, whilst that placed in the weak calcium chloride solution weighed 17 grammes. In fact a solution containing 40 c.c. of 1 $^0/_0$ solution of calcium chloride to 360 c.c. of distilled water swells the laminaria only to little more than double its dry weight, whilst laminaria in distilled water increases from five to seven times its dried weight.

The checking effect then of tap water on imbibition is obviously due to the lime contained in the tap water.

Tap water not only lessens the imbibition, but on removing the laminaria from the tap water, drying it, and then immersing it in distilled water, the tap water (lime) effects persist, and the laminaria swells only as much as it did in tap water; whereas a piece of laminaria steeped first in distilled water for some days, and then dried and immersed a second time in distilled water, swells as much or nearly as much as when for the first time steeped in distilled water.

Calcium chloride produces the same effect. I took four pieces of laminaria each weighing 4·8 grms. One piece I put in distilled water, another in 395 c.c. of distilled water with 5 c.c. of 1 $^0/_0$ calcium chloride solution, a third in 390 c.c. distilled water with 10 c.c. of calcium chloride solution, and the fourth in 380 c.c. of distilled water containing 20 c.c. of calcium chloride solution. On the seventh day the first weighed 22·35 grms., the second 17·25 grms., the third 14·3 grms., and the fourth 11·5 grms. After drying, the pieces of laminaria weighed respectively 3·3 grms., 3·6 grms., 3·3 grms., and 3 grms. Steeped in distilled water for seven days the pieces weighed respectively 19·5 grms., 12·3 grms., 7·5 grms., 6·25 grms.; so that the pieces of laminaria during the second immersion with distilled water imbibed less than during first immersion when the pieces were treated with lime solution. Thus the lime effects not merely persisted, but were even more marked in the second than in the first immersion.

Frequent repetitions of this experiment always yielded the same result.

We have just seen that laminaria immersed in water containing lime loses much of its absorptive power. If however the laminaria is dried and put in 2 $^0/_0$ chloride of sodium solution it regains much of its absorptive power so that when dried and immersed in distilled water it swells almost as much as it would had it never been treated with lime solution, as the following table shows:

Composition of fluid	Distilled water	5 c.c. calcium chloride solution in 400 c.c. distilled water	10 c.c. of calcium chloride solution in 400 c.c. distilled water	20 c.c. of calcium chloride solution in 400 c.c. distilled water	40 c.c. of calcium chloride solution in 400 c.c. distilled water	80 c.c. calcium chloride solution in 400 c.c. distilled water
Dried weight	3·65 grms.	3·65 grms.	3·65 grms.	3·65 grms.	3·65 grms.	3·65 grms.
On 10th day weighed	20·7 "	14·1 "	11·5 "	8·5 "	8·15 "	6·3 "
Were then dried and weighed	2·35 "	2·35 "	2·35 "	2·3 "	2·35 "	2·35 "
Then placed in distilled water and on 10th day weighed	18·8 "	7·4 "	6·3 "	6·1 "	6 "	5·3 "
Then again dried and weighed	2·3 "	2·3 "	2·3 "	2·3 "	2·35 "	2·3 "
Placed in 2 °/₀ saline and on 12th day weighed	13·9 "	8·6 "	7·9 "	11·5 "	7·1 "	5·8 "
Then dried and weighed	2·3 "	2·3 "	2·3 "	2·2 "	2·3 "	2·2 "
Then all placed in distilled water and on 9th day weighed	18·7 "	14·3 "	14·3 "	12·6 "	12·5 "	10·4 "
Weight of dried laminaria	4·8 "	4·8 "	4·8 "	4·8 "		
On 7th day of immersion weighed	22·35 "	17·25 "	14·3 "	11·5 "		
Were then dried and weighed	3·3 "	3 "	3·3 "	3·6 "		
Were then placed in distilled water and on 8th day weighed	19·5 "	12·3 "	7·5 "	6·25 "		
Were then dried and weighed	2·7 "	2·95 "	2·75 "	2·9 "		
Then all placed in 2 °/₀ saline and weighed on 9th day	9·8 "	9·3 "	7·4 "	7·3 "		
Were then dried and weighed	2·9 "	2·9 "	2·9 "	3 "		
Were then all placed in distilled water and on 9th day weighed	21·8 "	20·3 "	19 "	18 "		

It is sufficient to transfer the laminaria from the lime solution to the saline solution, without drying it, in order to obviate the effect of the lime. Then on drying and putting the laminaria in distilled water it swells again freely.

Though calcium chloride greatly lessens the amount of absorptive power of dried laminaria, yet if a piece of it is first placed in distilled water till it swells to the fullest extent and is then transferred to a lime solution it undergoes no diminution of weight. Lime greatly restricts the swelling, but it will not make swollen laminaria part with any of the water it has absorbed.

Other lime salts, like calcium chloride, restrain the swelling of laminaria. I steeped four pieces of laminaria cut from the same stem, each piece weighing 2·7 grammes, the first in 400 c.c. of distilled water, the second in 400 c.c. of ·2°/₀ solution of nitrate of lime, the third in 400 c.c. of ·4°/₀ solution of nitrate of lime, and the fourth in a saturated solution of sulphate of lime. On the sixth day they weighed respectively, (i) 14·2 grammes, (ii) 5 grammes, (iii) 4·5 grammes, (iv) 4·8 grammes.

Phosphate of lime is scarcely soluble in distilled water. Consequently it has much less action on laminaria. Of two pieces of laminaria, each weighing 3·8 grammes, one was placed in distilled water, the other in a saturated solution of tribasic phosphate of lime in distilled water. On the seventh day the first weighed 20·7 grammes, the second 19·5 grammes.

Lime water also restrains the swelling of laminaria. I cut four pieces of laminaria weighing 7·1 grammes each, the first I steeped in 400 c.c. distilled water, the second in equal parts of lime water and distilled water, the third in a solution consisting of one part of lime water in four of distilled water, and the fourth in a solution consisting of one part of lime water in eight of distilled water. On the 13th day they weighed respectively 38 grms., 19·2 grms., 20·2 grms., 25·2 grms. The pieces of laminaria were then dried and weighed, the dried weight of each piece being 5·3 grms.; and these were all placed in distilled water and on the 18th day weighed respectively 32·5 grms., 9·5 grms., 9·5 grms., 10·8 grms.

Solutions containing common salt with calcium chloride restrict the swelling of laminaria sometimes by one half; but on drying the pieces and putting them in distilled water they yet swell pretty nearly to the same extent as the laminaria that has not been subjected to a solution containing lime, as the following table shows:

	Distilled water	2°/0 saline with 40 c.c. calcium chloride solution in 400 distilled water	Same with 80 c.c. calcium chloride solution	Same with 120 c.c. of calcium chloride solution
Dried weight	2·1 grms.	2·1 grms.	2·1 grms.	2·1 grms.
After 16 days in solution	8·9 "	6·2 "	4·6 "	4 "
Then dried and weighed	1·3 "	1·5 "	1·4 "	1·3 "
Then all placed in distilled water and on the 8th day weighed	9·7 "	11·9 "	10 "	8·3 "

Laminaria being a sea-weed of course contains on drying a good deal of sodium chloride, and the question arises, What influence has this fact on the swelling of the dried plant when steeped in various solutions? Is the absorption due simply to diffusion of these salts with the corresponding osmosis of water? This certainly is not the explanation of the swelling, as numerous experiments show.

Two pieces of laminaria, each weighing 2 grms., were immersed, one in distilled water, the other in tap water, and on the fifth day they weighed respectively 11·9 and 6·7 grms. When thoroughly dried they weighed, one 1·9 and the other 1·3 grms.; they were then replaced as before, the first in distilled water and the second in tap water, and six days afterwards they weighed respectively 11 and 3·7 grms. They were a second time dried and each weighed 1·25 grms. and both were placed in distilled water, and on the second day the first weighed 9·15 grms. and the other only 2·95 grms.

In another experiment I put a piece of laminaria weighing 7·05 grms. in distilled water, and in eighteen days it weighed 33·9 grms. After being dried it weighed 5·7 grms., and a second time was immersed in distilled water, and in seventeen days it weighed 29·8 grms. Again it was dried and weighed 4·1 grms.; it was immersed a third time in distilled water, and on the sixteenth day it weighed 26·7 grms.

The fluid containing the laminaria, as I have said, was changed daily. On the first and second days the distilled water gives distinct traces of chloride when tested with silver nitrate solution, but on the third or following day the fluid contains no chloride. Hence I conclude that all the chloride of sodium has diffused out and yet swelling afterwards goes on; and on drying the laminaria and again immersing it in distilled water, it swells as rapidly and as—if we take into consideration the loss of weight after each immersion—freely as at first.

Has the sodium chloride any influence on the swelling? In the previous experiments we find that laminaria steeped in distilled water swells more rapidly than laminaria in solution of common salt, but though in the saline solution the plant swells more slowly still after a time it catches up the weight of the laminaria in distilled water. This fact suggests that the salt in the dried laminaria diffuses more quickly with distilled water than with saline solutions, causing a more rapid osmotic current, and thus accelerates the swelling; but this view is discountenanced by the fact that a piece of laminaria immersed in distilled water, dried, and again immersed in distilled water, generally swells more rapidly the second time when all the salt has been diffused out by the first immersion.

The previous experiments then show that sodium chloride and potassium chloride retard but do not lessen the amount of fluid taken up, and that therefore the diminished absorption by laminaria in tap water is not due to its sodium and potassium salts.

On the other hand minute traces of a calcium salt greatly restrict the swelling of laminaria and this persistently, for on drying a piece placed in distilled water containing lime and then immersing it in distilled water, it swells much less than if in the first instance put into distilled water.

This, I think, shows clearly that lime unites with some material in the laminaria; otherwise, treated with distilled water it should diffuse out and leave the laminaria to be acted on by the distilled water.

Sodium chloride however largely antagonizes this effect of lime, for if a piece of laminaria which has been steeped in a lime solution, and so has swollen but little, is placed in a 2°/₀ solution of sodium chloride, when dried and again put into distilled water it will swell as much almost as a fresh piece of laminaria.

Whilst lime salts will restrain the swelling of dried laminaria, yet if it is immersed first in distilled water till fully swollen the addition of lime will fail to lessen the amount of swelling.

I have shown that the swelling depends not on osmosis of salts; indeed the osmotic equivalent of common salt or lime salts is far too small to explain this prodigious swelling.

The swelling must then be due to the imbibition of water by the bassorin (algin), and this imbibition is evidently greatly restrained by lime salts. The following experiment confirmed this conclusion.

"Algin, a new substance obtained from some of the commoner species of marine algæ[1]," contains nitrogen in addition to carbon, hydrogen and oxygen, is insoluble in water, but becomes very soluble on the addition of a little alkali. Dilute hydrochloric acid, dilute nitric acid, indeed all the mineral acids coagulate it. Lime water, baryta water, salts of calcium and strontium give white precipitates.

It can be obtained by immersing the fronds of laminaria in water containing a little alkali, by preference sodium carbonate. The whole plant becomes disintegrated and presents a gelatinous mass consisting of a thick glutinous gummy liquid containing the cellular fabric of the plant completely broken up. It contains 2 °/₀ of algin. This can be separated by heating and filtering through coarse linen sacking.

The swelling of dried laminaria is chiefly due to the imbibition of water by this substance.

Algin is sold in thin paper-like sheets, which I used in carrying out the following experiments.

I placed three pieces of the algin sheet weighing ·33 grms. respectively in the following fluids:—400 c.c. distilled water; 400 c.c. distilled water with 4 c.c. of 1 °/₀ solution of calcium chloride; 400 c.c. distilled water with 8 c.c. of 1 °/₀ solution of calcium chloride.

These different specimens at once displayed a difference which day by day increased. In distilled water the algin rapidly swelled and became indistinguishable. In the solution containing the smaller quantity of lime the algin swelled considerably, but always could be detected as a thick fog at the bottom of the vessel.

In the solution containing the larger quantity of lime the algin hardly swelled at all, and we could throughout detect the shape of the immersed pieces.

This experiment, somewhat modified, I repeated by steeping pieces of algin weighing ·33 grms. respectively in the following fluids—400 c.c. distilled water, 400 c.c. tap water, 400 c.c. distilled water containing 8 c.c. of calcium chloride solution, 400 c.c. distilled water containing 16 c.c. of chloride of calcium 1 °/₀ solution.

In three days' time the algin in each case was much swollen and appeared as a mist or cloud at the bottom of the vessel. In the first the mist seemed to melt away into the surrounding medium. In the tap water the mist had a well-marked line of demarcation. In the third the line of demarcation was still more distinct, and in the fourth this line of demarcation was still more defined.

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[1] E. C. C. Stanford, F.C.S. *Pharmaceutical Journal*, June 9, 1883.

Lime then lessens the amount of fluid that algin can imbibe, probably by combining with the algin.

Cress seeds when placed in water become surrounded by a transparent insoluble zone of mucilaginous matter; and the swelling of this mucilaginous envelope is much greater when the seeds are put into distilled water than in distilled water containing minute quantities of calcium chloride.

These are instances of the effect of lime to lessen the swelling of a lowly endowed "degradation compound" of vegetable tissue. I will now give an instance of the influence of lime on an animal product.

In distilled water the gelatinous investment of the ova of frogs quickly swells, increasing for three or four days, at which time it is four to six times larger than the investment of ova in tap water. As it swells it becomes less apparent whilst the ova are in water, till at last it can be seen only by transmitted light, though of course the investment is apparent enough when the water is poured off the ova. As the gelatinous investment swells it becomes less tenacious, and after a few days is very easily torn. With the swelling the cloudiness or haziness of the investment diminishes and there is often noticed a halo immediately round the ovum, and outside that the glutinous matter is quite translucent.

The addition of a small quantity of calcium chloride to distilled water, as one part in 5000 or less, checks the swelling of the gelatinous envelope so that it does not become larger than when treated with tap water.

I find that sodium bicarbonate added to distilled water also lessens the swelling of the gelatinous envelope, but in a less degree than the addition of calcium chloride; for instance, one part of sodium bicarbonate in 5000 parts of distilled water failed to prevent the swelling of the gelatinous envelope beyond the point produced in tap water. But one part of sodium in 2500 parts of distilled water restricted the swelling to the degree produced in tap water.

I find too that 4 c.c. of $\cdot 6\,{}^{0}/_{0}$ saline to 100 c.c. of distilled water prevents the swelling of the gelatinous envelope more than occurs in tap water.

The swelling of the gelatinous envelope of frogs' ova when immersed in distilled water is far greater than can be explained by diffusion and osmosis and must be due to imbibition, and here we have another instance of the controlling influence of salts on imbibition.

I think then it is probable that the death of fish in distilled water is due not only to osmosis of the cells but to imbibition by the material which unites the cells to each other.

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FURTHER EXPERIMENTS REGARDING THE INFLUENCE OF SMALL QUANTITIES OF LIME POTASSIUM AND OTHER SALTS ON MUSCULAR TISSUE. By SYDNEY RINGER, M.D., F.R.S., *Professor of Medicine at the University College, London.* (Plate X.)

During the last year I have employed for experiments on the heart phosphate of lime in place of chloride of calcium in the composition of an artificial circulating fluid. Messrs Hopkins and Williams prepare the phosphate of lime by dissolving pure calcium chloride and adding a solution of rhombic phosphate of soda and well washing the precipitate.

Dr Graham, Professor of Chemistry at University College, who has kindly examined this phosphate, finds that it consists mainly of tricalcic phosphate with a trace of soluble phosphate and a very minute trace of free hydrochloric acid. The salt gives a decided acid reaction to test paper, the acidity being mainly due to the soluble phosphate. I add this phosphate of lime to ·6 °/₀ saline solution and allow it to stand till the saline becomes saturated and draw off the supernatant fluid with a syphon and refill the bottle with saline solution. I employ two forms of circulating fluid. One is composed of this phosphate of lime saline[1] each 100 c.c. containing 1 c.c. of 1 °/₀ solution of potassium chloride. The other contains in addition to each 100 c.c. 1 c.c. of 1 °/₀ solution of sodium bicarbonate. Either of these solutions will sustain good spontaneous contractions for several hours; even at the end of four hours with a vigorous heart the contractions are very little smaller than at the beginning of the experiment.

The solution containing sodium bicarbonate is, I think, rather more efficient. A smaller quantity of potassium chloride, i.e. ·5 c.c. of 1 °/₀ solution to 100 c.c., also makes a good circulating fluid and in some respects is better than the solution containing more potassium chloride, for the stronger solution (that containing 1 part of potassium chloride, to 10,000 parts) produces a very prolonged staircase of beats after only a short diastolic pause.

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[1] In this communication, phosphate of lime saline means ·6 °/₀ saline saturated with phosphate of lime.

This phosphate of lime circulating fluid I find very superior to a circulating fluid containing calcium chloride; moreover it is equally serviceable all the year round, whilst the fluid containing calcium chloride is much less efficient during some parts of the year than at others, succeeding much better in the autumnal and early winter months than in January and the subsequent months.

It is interesting to observe that though circulating fluid with phosphate of lime gives a slight acid reaction to delicate blue litmus paper it will sustain contractility for hours.

The action of phosphate of lime in sustaining the heart's contractions led me to test its influence in sustaining the contractility of skeletal muscles at rest by means of comparative experiments with saline and other solutions. In each series I used the muscles of the same frog and generally employed only the gastrocnemius muscles; then of course there were only two experiments in each series; but sometimes I used also the tibialis anticus or some of the thigh muscles. I placed the muscle in 500 c.c. of the testing fluid and renewed this twice and tested contractility by faradic excitation, thrice daily.

I first compared in December the effect of ·6 °/₀ saline solution with ·6 °/₀ saline saturated with phosphate of lime; also with the effect of phosphate of lime saline and each 100 c.c. containing 1 c.c. of 1 °/₀ solution of KCl. In nine experiments with saline solution contractility persisted on an average 27 hours.

In ten experiments with phosphate of lime saline contractility persisted on an average for 40 hours.

In twelve experiments with phosphate of lime saline containing potassium chloride, the average persistence of contractility reached 54 hours.

During the next month, namely in January, I repeated these experiments, but placed the whole posterior limb, stripped of its skin, in the solution and tested the excitability of the gastrocnemius thrice daily.

In five experiments with saline solution contractility persisted on an average 53 hours.

In five experiments with phosphate of lime saline contractility persisted on an average 87 hours.

I next compared the effect of saline solution containing sodium bicarbonate adding 2 c.c. of 1 % solution of sodium bicarbonate to each 100 c.c. of saline solution.

In six experiments with saline solution contractility persisted on an average 62 hours.

In six experiments with saline solution containing sodium bicarbonate contractility persisted on an average 84 hours.

In all the foregoing experiments the muscles were kept in a room artificially heated. In the following experiment the muscles were kept out of doors during very cold weather. In saline solution contractility persisted 130 hours. In saline solution containing sodium bicarbonate contractility persisted 152 hours.

I next compared the influence of saline solution, each 100 c.c. containing 2 c.c. of 1 % solution of sodium bicarbonate, with that of phosphate of lime saline.

In three experiments with saline solution and sodium bicarbonate contractility persisted on an average 94 hours.

In three experiments with phosphate of lime saline contractility persisted on an average 113 hours.

In two experiments when the vessels were kept out of doors during very cold weather contractility persisted on an average in the saline and sodium bicarbonate solution 148 hours, in phosphate of lime saline, 160 hours.

I next compared the effect of phosphate of lime saline with phosphate of lime saline containing in each 100 c.c. 1 c.c. of 1 % solution of potassium chloride.

In nine experiments with phosphate of lime saline contractility persisted on an average 77 hours.

In nine experiments with phosphate of lime saline containing potassium chloride contractility persisted on an average 87 hours.

I next compared the effect of phosphate of lime saline, each 100 c.c. containing 1 c.c. of 1% solution of potassium chloride, with phosphate of lime saline each 100 c.c. containing 1 c.c. of potassium chloride and 1 c.c. of 1% solution of sodium bicarbonate.

In six experiments with phosphate of lime saline containing potassium chloride contractility persisted on an average 77 hours.

In six experiments with the same solution containing sodium bicarbonate contractility persisted on an average 80 hours.

In the foregoing experiment the vessels were kept in a warmed room but in the following experiments the vessels were kept out of doors during very cold weather (January).

In six experiments with phosphate of lime saline, each 100 c.c. containing 1 c.c. of KCl solution, contractility persisted on an average 129 hours.

In six experiments with the saline solution plus 1 c.c. of sodium bicarbonate contractility persisted on an average 140 hours.

I made some more experiments in March with saline and phosphate of lime in saline and with saline compared with saline (200 c.c.) containing 4 c.c. of $1^{0}/_{0}$ solution of calcium chloride.

In thirteen experiments with saline the contractility persisted on an average 66 hours.

In thirteen experiments with saline containing phosphate of lime contractility persisted on an average 78 hours.

In fourteen experiments with saline contractility persisted on an average 65 hours.

In fourteen experiments with saline containing calcium chloride, contractility persisted on an average 71 hours.

In the latter half of February and in March I made some further comparative experiments with saline and saline containing sodium bicarbonate. Of six experiments in February with saline contractility persisted on an average 40 hours.

In six experiments in February with saline containing sodium bicarbonate contractility persisted on an average 42 hours.

In March in two experiments with saline contractility persisted on an average 20 hours, with saline containing sodium bicarbonate 23 hours.

We see then that these solutions in respect of their efficiency to sustain contractility stand in the following order. Saline solution is the least effective, next follows saline containing sodium bicarbonate, then phosphate of lime saline, next phosphate of lime saline containing potassium chloride. Phosphate of lime saline containing potassium chloride and phosphate of lime saline containing potassium chloride and sodium bicarbonate sustain contractility about an equal time. Phosphate of lime saline sustains contractility longer than saline solution containing sodium bicarbonate.

Season however appears to influence the effect when sodium bicarbonate is added to saline, for in the mid-winter months the sodium bicarbonate sustained contractility to a considerable extent, whilst in the latter part of February and in March, it lost its sustaining effect.

Now this being the order of their efficiency as circulating fluids for the heart, it would appear that lime and potash salts are as necessary to the metabolism of muscle at rest as to the metabolism occurring during a muscular contraction.

Another circumstance however explains in part the efficacy of lime and potassium salts in sustaining contractility in quiescent muscle.

I noticed in the muscles placed in saline active contractions. This was much more apparent in muscles that were partially detached, and especially noticeable in the sartorius when torn from the rest of the muscles and attached only by its lower end. Now these contractions must consume the energy of the muscles and hasten exhaustion and the advent of rigor mortis.

In February I commenced a further series of experiments regarding the effect of saline solution and saline solution containing other salts to produce contractions.

Since I made these observations I find that Biedermann[1] has made similar experiments with results quite in accord with mine. Our methods however differed in some respects, and I recount here certain additional experiments to those he performed.

In his experiments he employed the sartorius of a strongly curarized frog. He suspended the muscle in a solution consisting of a litre of water, 5 grms. NaCl, 2·5 grms. Na^2HPO^4, and 0·4 grms. Na^2CO^3, and in most of his experiments he kept the temperature below 10° C. He obtained contractions in all respects like those described in my experiments, but I employed all the muscles of the thigh cut into thin ribands and attached only at the knee. I did not curarize the frog and I placed the limb in simple saline. Biedermann took no tracings of the contractions.

It is obvious therefore that the alkaline soda salts do not play so important a part in the production of these contractions as Biedermann supposes. I shall shew that phosphate of soda or caustic soda added to saline increases somewhat the frequency and the strength of the contractions. As I have said though working under different circumstances I obtain the same results, so well described by Biedermann.

We both find that temperature strongly influences these contractions, these being more frequent and more vigorous at a temperature between 20 and 30° C. than below 10° C. At a low temperature the contractions become somewhat rhythmical. There occur long pauses free from contractions, varying from ten seconds to two or three minutes, and then a series of contractions take place of much the same strength and separated by about the same intervals. Occasionally the beats for a minute or two are quite rhythmical.

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[1] *Wien Sitzungsbericht.* Bd. LXXXII. 1880, p. 257.

I cut off both hind limbs of a frog through the hip joint and after stripping off the skin I cut the thigh muscles into thin ribands, and left them attached only at their lower end close to the knee joint. One limb I suspended in 200 c.c. 6°/$_0$ saline solution, the other in a similar quantity of saline solution containing some other salt.

On placing the limb in saline solution the strips of muscles at once contract frequently, and one sees both massive and fibrillary contraction, i.e. the whole muscle or piece of muscle is contracted, and in addition one sees the contraction of fibrillæ merely. The contractions begin soonest and are most marked in the thigh muscles cut into thin bands. Later fibrillary contractions occur in the uninjured lower leg muscles causing twitching of the toes. These contractions persist often for hours and continue longer in the uninjured than in the thigh muscles cut into ribands.

As Biedermann noticed with his solution so I find that when the contractions have ceased they often recur on renewing the saline solution.

On removing the limb from saline, strong fibrillary contractions persist for some time, and when they cease the excitation of a contraction by the faradic current brings them back again to persist without any further stimulation for half a minute or even longer.

The muscles of the fellow limb placed in phosphate of lime saline solution twitch much less, and in some cases no contractions at all occur.

The addition of 1 c.c. of 1°/$_0$ solution of potassium chloride to each 100 c.c. of the testing fluid very slightly lessens the fibrillary twitching of the muscles in saline solution. The twitchings of the muscles in phosphate of lime saline solution are greatly lessened or even utterly arrested; but on replacing the muscles in their original fluid the twitching again increases, and on the further addition of potassium chloride the same effect follows as on the first addition.

I find that sodium bicarbonate like lime salts prevents the muscular twitching.

On Jan. 16 and 19 I prepared the two hind legs of a frog in the manner described. One (A) I placed in 200 c.c. saline, the other (B) in 200 c.c. saline solution containing 8 c.c. of 1°/$_0$ solution of sodium bicarbonate. Muscular twitching began at once in the limb placed in saline, but none occurred in the muscles placed in the saline and sodium bicarbonate solution. After half an hour I added 8 c.c. of sodium bicarbonate solution to the 200 c.c. saline solution and removed the other limb from saline and sodium bicarbonate solution and placed it in 200 c.c. saline. The addition of sodium bicarbonate to saline solution (A) lessened the movements, so that in thirty-three minutes the muscular twitching had almost ceased. The muscles of leg (B) removed to simple saline immediately began to twitch and both the partially detached (thigh) and entire (lower leg) muscles twitched vigorously.

I then removed (A) from the saline and sodium bicarbonate solution and replaced it in saline, when the muscular twitchings immediately increased and became quite vigorous in both the partially detached and in the lower leg muscles (tibialis anticus and gastrocnemius).

In two hours and a half the twitching in both limbs had almost if not quite ceased.

I next tested in the same way the action of a weaker solution of sodium bicarbonate 4 c.c. to 200 c.c. of saline, and I found that whilst the twitching of the partially detached muscles of the thigh and the uncut gastrocnemius and tibialis anticus etc. of the limb placed in saline twitched vigorously, the slightest movements only occurred in the fellow limb placed in saline and sodium bicarbonate solution.

I next tested a weaker solution, adding 2 c.c. of sodium bicarbonate $1^{0}/_{0}$ solution to 200 c.c. saline (1 in 10,000 parts of sodium bicarbonate), and found that this minute quantity lessened the twitchings, which though marked were decidedly less than the twitchings of the fellow limb placed in simple saline solution.

In February and again in March I repeated these experiments with sodium bicarbonate solution, adding to the 200 c.c. saline 4 c.c., 6 c.c., 8 c.c. and 12 c.c. of $1^{0}/_{0}$ solution of sodium bicarbonate. I made nine comparative experiments and found that the sodium bicarbonate did not influence the movements at all, or if it had any effect it slightly increased the contractions, and I found that contractility tested by a faradic current persisted about the same time in the muscles placed in saline as in those placed in saline plus sodium bicarbonate. The average of six experiments with saline being 40 hours and of six experiments with saline and sodium bicarbonate being 42 hours.

I find that small doses of potassium chloride 1 c.c. to 4 c.c. of $1^{0}/_{0}$ solution added to 100 c.c. of saline somewhat lessen the contractions. These quantities added to phosphate of lime saline solution at once check any movements, though, as I have said, phosphate of lime without the aid of potassium chloride generally prevents any movements.

The addition of potassium chloride whilst it somewhat restrains the movements has but little effect in sustaining contractility, for in five experiments with saline contractility persisted on an average 63 hours.

In five experiments saline solution containing 4 c.c. of potassium chloride to 100 c.c. sustained contractility on an average 69 hours.

These experiments then confirm the conjecture that salts in part sustain contractility by preventing or lessening twitching of the muscles, for phosphate of lime in saline greatly lessens or even prevents them, and the salt greatly prolongs the period during which contractility persists.

Sodium bicarbonate too in the mid-winter months greatly lessens twitching but to a less extent than phosphate of lime, and sodium bicarbonate also sustains contractility though for a shorter period than phosphate of lime; whilst a little later in the year sodium bicarbonate loses its influence over the twitching and loses also power to sustain contractility.

Further potassium chloride added to saline controls the contractions but slightly and it has but little power to sustain contractility.

Temperature strongly influences the contractions.

The contractions are most active between 20° and 25° C. Below 10° C. they diminish much and at 8° C. they almost stop, becoming not only less frequent but weaker. The contractions are especially active as the temperature of the fluid is rising. At 35° to 38° the movements quickly cease and the muscle passes into rigor.

Saline made with tap water prevents these twitchings, as the following experiment proves.

On Jan. 20 I placed one posterior extremity in 200 c.c. distilled water saline, the other in 200 c.c. tap water saline. The muscles in the distilled water began to twitch in three minutes and in ten minutes became quite brisk, the lower leg muscles quivering actively as well as the partially detached muscles of the thigh. The twitchings continued all day and were quite energetic eight hours from the beginning. The muscles in tap water saline did not twitch at all.

Contrary to what one would expect distilled water causes few or no twitchings.

On Jan. 20 I placed one hind limb of a frog in 200 c.c. distilled water saline and the other in distilled water. Active twitching persisted in the muscles in saline solution three hours, but had ceased in five hours and a half. In the muscles in distilled water one twitch occurred five minutes after the immersion but no other movement, and all the muscles soon passed into firm rigor.

I next took tracings of the sartorius in various solutions. I carefully separated the sartorius from the pelvis and left it attached only to the tibia. I cut off the greater part of the thigh and lower leg and then fixed the preparation and placed it in the testing fluid, attaching the free end by a ligature to a lever supported by a fine indiarubber thread against which the contracting muscle had to act.

In Fig. 1 (Pl. X.) I give a tracing from a sartorius placed in $\cdot6\,{}^{0}/_{0}$ saline. The contractions are considerable and frequent. There is also persistent spasm occurring in the form of a wave.

All muscles however do not contract to the extent shown in this figure, the sartorius of one leg often contracts considerably more than the sartorius of the fellow leg. Indeed in some instances there are no contractions. Where they are slight they generally cease in a few minutes. When however the contractions are marked, they often persist half an hour to an hour.

The persistent spasm or tonic contraction too varies in amount. It is generally most marked when the rapid contractions are strongest. The tonic spasm gradually grows less and ceases in a few minutes, even when the contractions persist longer.

Sometimes it uniformly grows less; in other cases, as shown in the tracings, it relaxes a little and then returns, so that waves appear in the trace. Even when there are no rapid contractions, tonic spasm often occurs and slowly relaxes.

As the muscle grows weak pauses occur of variable length, that is of a few seconds to several minutes, followed by a series of rhythmical beats, and in some instances the beats are about the same strength, and separated from each other by the same interval; they are indeed perfectly rhythmical. In other cases the rhythm is somewhat irregular. With these contractions there often occurs some persistent spasm, and with some of the contractions relaxation is slow, see Fig. 2, *A, B, C.* These rhythmic contractions too frequently take place when the rapid contractions are much restrained by adding to the 200 saline solution 4 c.c. $1\,{}^{0}/_{0}$ solution of calcium chloride. See *D.*

As the exhaustion progresses the non-beating periods increase in duration, and the number and strength of the following beats diminish.

At present I am unable to explain in all cases the different amount of contraction in different experiments with saline; for instance, when the sartorius of one leg contracts much more than the sartorius of the other leg. I think that in some cases exposure of the muscle for twenty to thirty minutes increases the amount and frequency of the contraction; at least it commonly happens that the sartorius last tested contracts much more than the other first tested sartorius.

The condition of the frog no doubt greatly influences the contractions. In exhausted muscles a stronger minimal stimulus is required to produce a contraction. As the stimulus from saline solution is evidently feeble in a weak frog, we should naturally expect weak contractions or no contractions at all, and this indeed is the case.

As with the former experiment, so with the graphic method, I find temperature greatly influences the frequency and extent of the contractions. With a temperature about 10° C. the contractions are infrequent and not very strong, but on raising the temperature to 25° or 30° the contractions grow much greater and more frequent. With the temperature about 10° C. or lower, the contractions are very similar in frequency and strength to those occurring at a higher temperature, after more frequent and energetic contractions have somewhat exhausted the muscle. There occur pauses without any contractions, varying from a few seconds to several minutes; and then a series of contractions follow, the interval between these being in some instances much the same, in other instances varying a good deal and then again a pause occurs.

After the contractions have grown less frequent and weaker, by renewing the saline solution the contractions again improve. Biedermann noticed the same effect with his alkaline saline solution, and attributes the weakening of the beats to the production of contraction products which diffuse into the fluid and depress the function of the muscle.

Mere alteration in the percentage amount of salt influences the contraction, as the following experiment shows. On April 13 I placed the sartorius in ·5 $^0/_0$ saline solution. For the first three minutes only the faintest contractions occurred. They then for two or three minutes became moderately strong and again grew weak. I then added 25 c.c. 2 $^0/_0$ solution of salt to 200 c.c. of ·5 $^0/_0$, which much increased the activity of the contractions. I then returned to ·5 $^0/_0$ solution and in about a minute the contractions became much weaker. I then added 25 c.c. of 2 $^0/_0$ solution to 150 c.c. ·5 $^0/_0$ solution and the contractions became greatly increased. I added another 25 c.c. of 2 $^0/_0$ solution and still further increased the contractions. Again I returned to 150 c.c. 5 $^0/_0$ solution and the contractions greatly lessened, occurring in paroxysms with quiescent periods of half a minute. I then added 50 c.c. 2 $^0/_0$ solution, and again greatly increased the frequency of the contractions. On returning to 150 c.c. 5 $^0/_0$ solution contractions ceased but returned for a short time on adding 50 c.c. of 2 $^0/_0$ solution of sodium chloride.

On the other hand, lessening the relative amount of NaCl, as by adding distilled water to the saline solution lessens or prevents these muscular contractions. For instance, reducing the ·6 $^0/_0$ to ·5 $^0/_0$ NaCl decidedly lessens the contractions. Here I will record one experiment showing the effect of dilution. I first took a trace with 200 c.c. ·6 $^0/_0$ saline solution, and obtained active contractions. On adding 50 c.c. distilled water, the contractions ceased and failed to return on adding another 50 c.c. and a third 50 c.c. distilled water. On returning to ·6 $^0/_0$ saline, slight contractions recommenced, and the further addition of 2 c.c. of 2 $^0/_0$ $BaCl^2$ induced active contractions.

I next tested the effect simply of distilled water on the contractions. I first took a trace with ·6 $^0/_0$ saline solution, and then replaced this by distilled water, when the contractions ceased at once, though in some experiments a few single strong contractions occurred at many minutes' interval. The distilled water however induced some persistent contraction, which passed away on the addition of saline, provided not too long a term had elapsed, before adding the NaCl, i.e. about eight to ten minutes.

When these contractions brought out by immersion in saline cease contractility still persists, and stimulating the muscle with a faradic current the coil, standing at 15 to 20, will excite good contractions. Moreover, the addition of substances like barium chloride at once developes active contraction; indeed barium chloride will produce contractions and persistent spasm much more marked than with saline at the beginning of the experiment.

Saline saturated with phosphate of lime greatly lessens the contractions. I have made many experiments, employing alternately with the same muscle saline solution and phosphate of lime saline solution, and always with the same result. With the entire sartorius however the difference is not so great between saline solution and phosphate of lime saline solution as it is with the muscles of the thigh cut into strips. Perhaps the difference depends on the time of year, for with the thigh muscles the difference is greater in January and February than in March; but the difference is of this kind, that with either solution the contractions in March are much less than in the earlier months.

I made twelve experiments to test also the effect of calcium chloride using 1 °/₀ solution. I added 2 to 4 c.c. of this to 200 c.c. of saline solution. After testing the effect of simple saline I added at once 4 c.c. of calcium chloride solution. In every instance I tried this solution it almost immediately to a great extent lessened the contractions, and in many instances all but arrested them, in some, quite stopped them. In several cases after a few minutes slight contractions returned but were much less than with saline at the beginning of the experiment. In eight of the experiments at one period after the addition of lime chloride the contraction became literally rhythmic, i.e. the contraction occurred at pretty equal intervals and in much the same force. The following short account of an experiment is a good example of the experiments. On April 5 the sartorius in saline contracted actively. The addition of 4 c.c. of $CaCl^2$ solution at once almost arrested the contractions which were rhythmic in character. I then returned to saline solution, which induced active contractions, and these were at once arrested on the addition of 4 c.c. of calcium chloride solution. Again I placed the muscle in simple saline and again active contractions occurred, which were at once arrested on adding 4 c.c. of 1 °/₀ solution of potassium chloride.

The addition of lime chloride arrests not only the rapid contractions but also removes the persistent contraction, so that the trace rises higher above the base line. These effects are shown in Fig. 3.

In many experiments made with potassium chloride I find its action is largely influenced by temperature. My experiments have been made generally with the fluid at from 10° to 16° C. Soon after adding 4 c.c. of 1 °/₀ solution of KCl to 200 c.c. saline solution the muscle strongly contracts, dragging down the recording lever far below the level reached even when the contractions are well marked with saline; the muscle then slowly relaxes, but before it has much elongated fresh contractions set in, or when at its maximum contraction frequent well marked contractions occur, and these cease and the muscle slowly relaxes, see Fig. 4. Then the muscle remains quiescent for a variable time, ten seconds to one or two minutes, when contractions like those recorded in Fig. 5 occur and again the muscle becomes quiet, or after a few minutes again contracts as shown in the figure. By degrees the contractions become less marked and at last merge into a single slight contraction which relaxes slowly. With some muscles only these slighter contractions occur throughout the experiment. With a higher temperature the addition of potassium chloride solution, just like calcium chloride at once arrests the contractions. 1 c.c. of 1°/₀ solution diminishes the contractions greatly, but 2 c.c. i.e. 1 part of KCl in 5000 well nigh stops them or stops them altogether, and on returning to simple saline solution the contractions recommence actively.

The following experiment is a good example of the influence of potassium chloride. On Feb. 26 a sartorius muscle placed in saline contracted energetically. To the 200 c.c. of saline I then added 2 c.c. of 1°/₀ solution of potassium chloride and the contractions all but ceased. Again I placed the muscle in 200 c.c. of saline solution and active contractions returned. I again added 2 c.c. of potassium chloride solution and again greatly restrained the contractions, yet a third time saline solution restored the contractions, and this final trial stopped the contracting. In the previous experiment the changes were made quickly, but after the addition of 4 c.c. of potassium chloride solution to 200 c.c. saline solution, if the effect is watched for a considerable time, it generally happens that after an interval of two or three minutes contractions return, but occur in groups, a long interval often intervening. The contractions are often very vigorous and rapidly repeated; these outbursts of contractions at long intervals grow weaker and then cease. In some cases the trace is in the form of an irregularly wavy line.

Potassium chloride then whilst modifying considerably the character of the contraction induces very marked contractions, and our previous experiments showed that potassium chloride added to saline solution does not sustain contractility. The difference between calcium chloride and potassium chloride is very striking, as the following experiment shows. On May 6 saline solution excited active contractions, which were at once cut short on adding 4 c.c. of 1°/₀ solution of calcium chloride to 200 c.c. saline. After six minutes I replaced the sartorius in saline and soon after added 4 c.c. of 1 °/₀ solution of potassium chloride. This produced the effect shown in Fig. 5. I added 4 c.c. $CaCl^2$ solution and at once stopped all contractions. In about four minutes I replaced the muscle in saline without inducing any strong contractions, as generally happens when saline solution replaces saline solution containing potassium chloride 1 part in 5000. Soon after I added 4 c.c. potassium chloride solution and again induced the usual potash effect, which was arrested again though more slowly by adding 4 c.c. of calcium chloride solution. I then again reverted to simple saline without inducing any contractions and again added 4 c.c. potassium chloride solution and induced well marked potash contraction. These were again stopped though not so rapidly as on the first occasion by adding 4 c.c. of calcium chloride solution. Again I returned to saline, then added potassium chloride solution and again excited well marked potash contractions, which calcium chloride once more arrested. This action of potassium chloride and the antagonism of calcium chloride I many times verified.

On March 20th I tested the action of 4 c.c. and 8 c.c. of 1°/₀ solution of sodium bicarbonate added to 200 c.c. of saline solution and found that the sodium bicarbonate rather increased the contractions, which became immediately considerably lessened on adding 2 c.c. of potassium chloride 1°/₀ solution. At this time of year I have found that sodium bicarbonate added to saline solution does not sustain contractility.

Thus the graphic method confirms the experiments in which the movements were estimated by sight: in most cases saline solution induces active contraction and persistent spasm (contracture or tonic spasm). Warmth greatly favours these movements, whilst cold approaching 0° C. retards or prevents them. If the contractions are restrained by cold, or even with a higher temperature when the contractions grow weak, they become rhythmical and occur in groups separated by long contractionless periods. Persistent spasm may occur without any rapid contractions, and whether with or without rapid contractions persistent spasm passes off in a few minutes. If they pass away without rapid contraction the trace takes the form of a slowly ascending line.

The addition of more NaCl to the ·6°/₀ saline increases the vigour and frequency of the contractions, whilst the dilutions of the saline with distilled water lessens and arrests the contractions. The replacing saline solution with distilled water arrests the contractions.

By adding calcium chloride 1 part to 5000 parts of saline solution these contractions are almost immediately lessened or arrested. In some instances weak contraction recurs after a few minutes. Sodium bicarbonate slightly increases the contractions in March. Potassium chloride greatly modifies the character of the contraction without lessening at certain temperatures to any marked extent the amount of contraction.

I will now record some experiments made with the wish to ascertain if the contractions depend on the direct effect of the solution on the muscle, or indirectly through its effect on the nerve, or whether the solution can induce contractions in both ways.

As saline excites active contractions in the sartorius separated from the limb with the exception of its lower tendon, if the saline acts through the nerves, it can do so only through its action on the terminations of the motor nerves in the muscle.

Biedermann in his experiments with strongly curarized frogs obtained contractions with his alkaline saline solution. I too have strongly curarized moderate sized frogs by injecting two doses at a few hours' interval of 1 c.c. of ·1% solution of curare solution and then amputated the posterior limbs, stripped off the skin and cut the thigh muscles into thin bands, and on immersing the limbs in ·6% saline strong contractions ensued. These experiments however appeared to me liable to error. In former communications I have already shown that many substances merely suspend and do not destroy function, and that their activity in this respect depends on the percentage dose, and this being reduced function returns. For instance, if to a circulating fluid enough potassium chloride is added to arrest the heart, by diluting the circulating fluid with fluid free from potassium chloride good contractions return. Curare again unless employed in very large doses simply suspends function and is gradually eliminated and the animal recovers. It occurred to me then as a feasible explanation that by immersing the curarized muscles into a large quantity of saline, the curare might get diffused out with return of function; the following experiment shows that this is precisely what occurs.

On March 26 I injected under the back of a frog weighing 34 grammes 1 c.c. of ·1% curare at 2 p.m. and again at 5 p.m. Next morning at 9 a.m. I cut off one leg and on applying a faradic current to the sciatic the coil being even at 0 did not excite any contractions; consequently its motor terminations were completely paralysed. I then placed the limb in 200 c.c. saline solution. In fifteen minutes the coil standing at 12 faradisation of the sciatic nerve excited slight quiverings of the toes. In thirty minutes the quivering rather increased and continued to increase, and in an hour and a half the foot muscles contracted with the coil at 27. In an hour and three-quarters the foot muscles contracted vigorously with the coil at 27.

Here then the curare diffused out and nerves perfectly paralysed became competent to conduct weak excitations, but the process was slow, whilst with curarized muscles placed in saline solution strong contractions commence at once.

I have made further experiments on this question.

On Jan. 15 at 9 a.m. I injected a small sized frog with ·5 c.c. of ·1% curare solution and repeated the dose in the evening. Next morning at 9 a.m. I amputated both posterior limbs, tested the nerves and found they did not conduct, removed the skin and cut the thigh muscles into thin strips. One limb I placed in 200 c.c. saline solution the other in 200 saline solution containing 3 c.c. of ·1 $^0/_0$ curare solution. Brisk twitching immediately commenced in both limbs. The twitchings increased equally in both, and involved the lower leg muscles as well as the cut thigh muscles. In about an hour the muscular contractions grew much less in the cut thigh muscles, and in two hours were much less also in the lower leg muscles.

It appears then that the saline solution excites twitchings by its direct action on the muscular tissue, unless the quantity of curare added to the saline is very small, and permits the curare in the nerves to diffuse out; but this explanation can hardly pass, for the twitchings began immediately the limb was immersed in the curarized saline and were as active as those of the limb placed in the non-curarized saline.

I next tested the effect of curare on tracings of the sartorius.

On March 26th I injected at 2 and 5 p.m. 1 c.c. of ·1 $^0/_0$ solution of curare and next day at 9 a.m. I tested the sartorius. The sciatic nerve of the limb failed to conduct when strongly faradised. The sartorius contracted vigorously when placed in saline. I then added 4·5 c.c. of curare solution to the 200 c.c. of saline; contractions continued for 35 minutes, when the experiment was discontinued. This experiment strongly supports the view that saline induces contractions by its direct action on the muscle, for after the experiment the sciatic still failed to conduct the strong excitations of a faradic current to the muscle with which it still remained connected.

I next tested the action of the other members of the lime group, namely, strontium and barium. I used 1 $^0/_0$ solution of strontium chloride, 2 $^0/_0$ solution of barium chloride.

In a former communication[1] I have shown that in the composition of an artificial circulating fluid for the frog's heart strontium chloride can fairly well replace calcium chloride, whilst I showed that barium chloride in its action is far removed from both these substances and cannot in any degree replace them in a circulating fluid. Barium induces in the ventricle a great deal of persistent spasm and causes the muscular coat of blood-vessels to contract strongly.

As with the heart muscle so with skeletal muscle, strontium is very similar to lime, whilst barium is far removed from both. Thus strontium chloride like lime chloride added to saline solution in the proportion of 1 part to 5,000 lessens the contractions which saline solution induces. Barium chloride on the other hand when added in the same proportions to saline solution acts quite differently. If added at the beginning of the experiment it has no effect on the contractions or may slightly increase them and induce slight persistent spasm. If however the sartorius is first subjected to the action of saline solution for ten to twenty minutes and the saline solution is replaced by fresh saline, then, on adding 2 c.c. of barium chloride to 200 c.c. saline solution, very active and powerful contractions occur, much greater than ensued with simple saline solution, and at the same time great persistent spasm is induced. The persistent spasm often relaxes a little and then again increases and thus assumes the form of waves. See Fig. 6.

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[1] *Practitioner*, August, 1883.

The absence of barium-effect before the renewal of the saline is due I suggest to the salts of potash and salts of lime, in the muscle or saline into which they have diffused, antagonizing for the greater part the barium salts; for if after replacing saline 4 c.c. to 6 c.c. of KCl 1 °/₀ solution are added barium effects are prevented; or if barium chloride is first added the induced persistent and rapid contractions are speedily arrested by adding potassium chloride or calcium chloride.

DESCRIPTION OF FIGURES.

Plate X. Fig. 1.

A. Sartorius in ·6 °/₀ saline taken March 20. It shows well marked and rapid contractions, also persistent spasm varying in amount and assuming the form of waves.

B. A sartorius trace in saline solution taken on Feb. 26. Here the rapid contractions are much less marked, but the wave-like persistent spasm is well marked.

C. Trace of sartorius in saline solution, the drum revolving more rapidly.

Fig. 2.

A. Effect of saline solution, temperature 10·5° C. fifty minutes from the beginning of the experiment.

B. Effect of saline solution, temperature of fluid 10° C., seventy minutes from the beginning of the experiment.

C. Trace with saline.

D. Trace with 200 c.c. saline to which 4 c.c. $CaCl^2$ 1 °/₀ solution was added, seven minutes after the addition.

Fig. 3.

A. Shows the effect of saline. At the cross 4 c.c. 1 °/₀ $CaCl^2$ were added to 200 c.c. saline.

B. The trace seven minutes after the adding of $CaCl^2$ solution, showing the slight return of the contraction. This return is much greater than usually occurs. At the end of fourteen minutes the contractions almost ceased, but returned again placing the muscle in saline solution.

Fig. 4.

Effect of adding 4 c.c. 1 °/₀ solution of KCl to 200 c.c. saline solution added at the point indicated by a cross.

Fig. 5.

Later effect of potassium chloride.

Fig. 6.

Showing the effect of adding 2 c.c. 2 °/₀ solution of $BaCl^2$ to 200 c.c. saline solution. Barium chloride added at the point indicated by a cross. At the end of eight minutes persistent spasm still persisted.

Fig. 0.

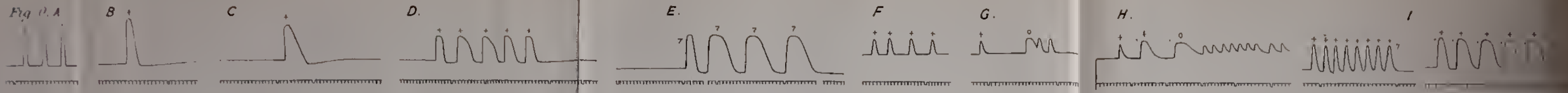


Fig. 0 a.

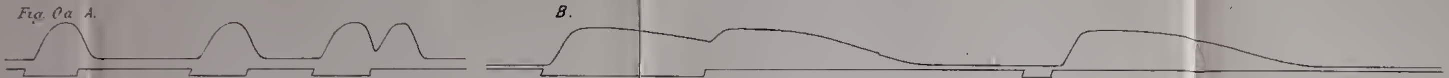
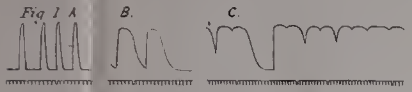


Fig. 1.



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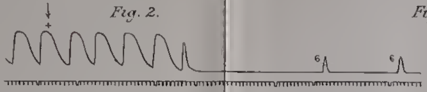

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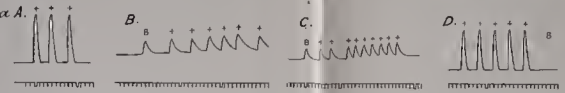


Fig. 3 a.

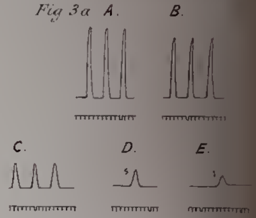


Fig. 3.

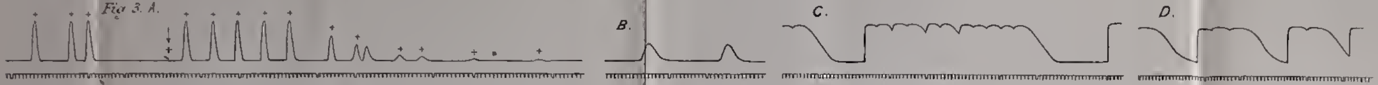
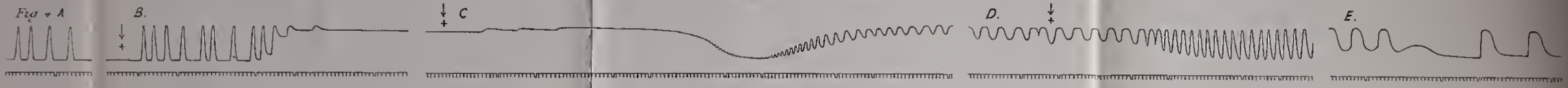


Fig. 4.



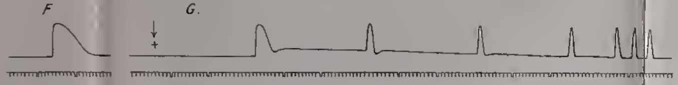


Fig. 5.

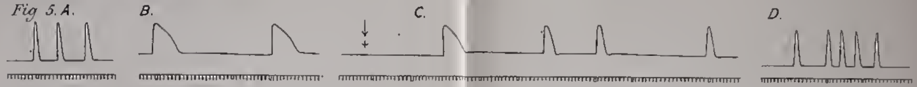


Fig. 4 a.

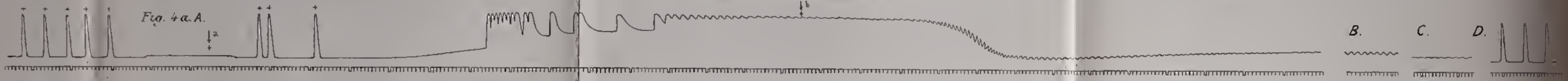


Fig. 6.

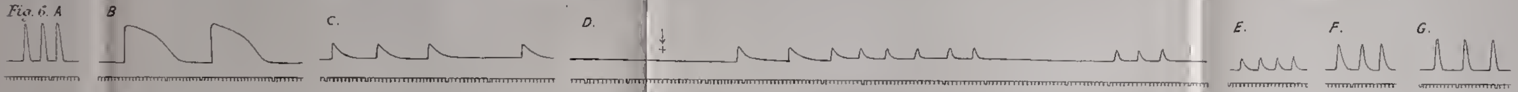
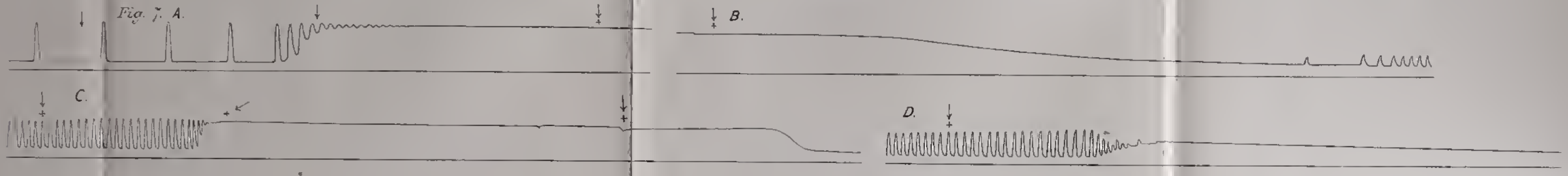


Fig. 7.



[*From the Journal of Physiology. Vol. VIII. No.* 1.]

REGARDING THE ACTION OF LIME POTASSIUM AND SODIUM SALTS ON SKELETAL MUSCLE. By SYDNEY RINGER, M.D., F.R.S., *Professor of Medicine at University College, London.*

During the months of May and June I made a series of experiments intended to ascertain whether minute quantities of calcium and potassium salts affect skeletal muscle as they do cardiac muscle.

I have already shown that an artificial circulating fluid free from organic substances does not, unless it contains a lime salt, sustain contractility in cardiac muscle, and that the contraction which occurs when the ventricle is fed with ·6 °/₀ saline solution containing a lime salt is abnormal, dilatation being greatly delayed unless the lime salt is partially antagonized by a small quantity of a potash salt. I find that ·6 °/₀ saline solution, saturated with phosphate of lime[1], antagonized by a small quantity of potassium chloride, will sustain for many hours the contractility of the frog's heart.

Although difference in function implies difference in chemical construction, still the difference in function between cardiac muscle and skeletal muscle is so slight, that it is fair to expect that substances which affect cardiac muscle will in a greater or less degree affect in a similar manner skeletal muscle.

Dr Brunton has already published some experiments made with the gastrocnemius muscle. He finds that "calcium and potassium salts affect skeletal muscle, as they do cardiac muscle." "Calcium in dilute solutions prolongs the duration of the contraction in the gastrocnemius of the frog. Potassium salts subsequently applied shorten the contraction[2]."

I am induced to publish the experiments recorded in this paper because the results I obtained differ in several respects from those obtained by Brunton.

I selected the sartorius, because, this being a very thin muscle, salts could more rapidly diffuse into or out of the muscular tissue and more readily influence the contraction.

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[1] This *Journal*, 1886.

[2] *Proceedings of Royal Society*, 1883.

I carefully separated the sartorius from the pelvis and dissected it from the other muscles, leaving it attached only by its lower end, and then cut off all the tissues except the bone into which the sartorius is inserted. I then placed the muscle in 200 c.c. ·6 % saline solution, fixing the lower tibial end, and applied the electrodes to the lower end of the muscle. The cut pelvic end I secured with a ligature and attached this to a recording lever suspended by a very thin piece of india rubber, which the contracting muscle stretched. I so arranged the apparatus that the ligatured muscle was merely suspended and not in any degree stretched.

To the saline solution I added the testing fluid, employing 1 % solutions of calcium chloride and of potassium chloride.

I excited the muscle by single induction shocks from a du Bois induction apparatus with a single Daniell's cell.

When the muscle is placed in ·6 % saline solution, rapid contractions occur as I have already described[1]. After a few minutes' immersion in saline solution and even before these contractions cease, on stimulating the muscle sufficiently to excite a fairly strong contraction, i.e. the coil standing at 6, there occurs a good deal of "contraction remainder." If the contractions are rapidly induced, this "contraction remainder" soon wears off, but returns if an interval of one or two minutes elapses between them.

The addition of a small quantity of calcium chloride, 1 part in 5000, entirely prevents in three to four minutes this "contraction remainder."

On frequently exciting the muscle placed in ·6 % saline solution, for instance thirty times in the minute, so as to induce moderate contractions, gradually increasing the strength of the stimulus as the contractions grow weaker, until the coil stands at *O*, I find that contractility becomes exhausted in an hour and a half to two hours.

In both these respects the effect of saline solution on skeletal muscle is very different from the effect on cardiac muscle. For saline solution induces in the heart no persistent contraction, nor anything like contraction remainder, and the contractions rapidly grow weak, the heart ceasing to beat in a few minutes and remaining in complete diastole.

The difference in the time contractility persists may probably be-explained in part by the difference in texture between cardiac and skeletal muscle. The spongy condition of the frog's heart allows the saline solution to come freely in contact with the muscular tissue and to diffuse out its lime and potash salts, whilst the more solid sartorius permits diffusion much more slowly. I have shown too that when serum-albumin is dialysed for several days, and then added to saline solution it sustains contractility in the frog's ventricle though the contractions are weak, and dilatation is much delayed, the fluid in this latter respect acting like a lime salt. On drying and incinerating the residue, the ash I find contains plenty of lime, therefore it is impossible to say whether the action of serum-albumin is due to the albumin or to the lime chemically combined with it. Anyhow the construction of skeletal muscle, i.e. its sheath and the fibrous septa between the muscular fibres would render diffusion of the albumin with its combined lime salt very slow and difficult; more so than in the spongy ventricle of the frog, the trabeculae moreover being covered by a very thin endocardium. With the frog's heart, fed with saline, when contractility ceases, the addition of a small quantity of a lime salt, such as calcium chloride or better calcium phosphate, restores good contractions; the addition of a lime salt under similar conditions does not restore the contractility of skeletal muscle.

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[1] This *Journal*, 1886.

Does this difference indicate a fundamental difference between skeletal and cardiac muscle? The difference may be due in part to the texture of the respective muscles—the condition of the sartorius being opposed to diffusion of albumin and inorganic salts from or to the muscle. For, although the addition of lime to saline will neither improve weakened contractions nor when lost restore them, if saline plus lime salt is used, then contractility persists much longer than if saline simply is employed. With saline containing a lime salt contractions in the sartorius will continue when excited thirty in the minute for six to eight hours, instead of, as with saline, an hour and a half to two hours. Still the diffusion rate does not explain all the difference between cardiac and skeletal muscle. For if large quantities of calcium chloride, after the skeletal muscle has become weak, are added to saline enough to affect the contraction in other ways, as for instance enough to remove any "contraction remainder," still no improvement in the contractions ensues. It is obvious then that if the lime will prevent "contraction remainder" it must have diffused into the muscular tissue and yet without strengthening the weakened contractions.

On adding even large doses of lime chloride to saline I am unable to prolong contraction in the sartorius or delay relaxation, in this respect differing from Brunton's results with the gastrocnemius, and differing too from the effect of saline and calcium chloride on cardiac muscle.

I first tested the action of 200 c.c. saline solution containing 4 c.c. of $CaCl^2$ solution. This prevented any contraction remainder. Then I added 2 c.c. of 10 % solution of $CaCl^2$, and subsequently another 2 c.c. and a third 2 c.c. without prolonging the contraction or retarding relaxation, but the contraction became a little weakened, showing that the lime had diffused into the muscular substance in sufficient quantity to produce this effect.

This difference between skeletal muscle and cardiac muscle cannot be explained by difference of construction, cardiac muscle of the frog permitting more rapid diffusion; for even small quantities of lime chloride (1 part in 5000 parts of saline), obviate contraction remainder. It is evident therefore that lime diffuses into the muscle, yet it does not affect the sartorius as it affects cardiac muscle.

The construction of the muscle and its influence in lessening diffusion of serum-albumin and its combined lime would explain the long-sustained contractility: but, if skeletal and cardiac muscle are alike as regards their behaviour to salts, then the relaxation of the sartorius after a contraction should be delayed either by the effect of albumin itself or by the action of its combined lime; but as I have said there occurs with the sartorius no delay of relaxation when the muscle is placed in saline solution or when moderate quantities or even large quantities of lime salt are added. I find too that injecting 2 c.c. of 10 % $CaCl^2$ solution under the skin of the back of a moderate-sized frog, a dose sufficient to greatly weaken the animal, does not delay the contraction of the sartorius, though the lime prevents any contraction remainder; but it may be that the potash salt in the blood of the frog is sufficient to prevent lime effects.

I next tested the action of sodium bicarbonate. The addition of from 4 to 6 c.c. to 200 c.c. of saline solution slightly lessens contraction remainder, but is greatly inferior in this respect to calcium chloride.

Potassium chloride differs from lime salts and from sodium bicarbonate in respect of its influence on contraction remainder, for 1 part of potassium chloride in 5000 parts of saline solution greatly increases contraction remainder, whilst the addition of calcium chloride obviates this potassium effect.

Potassium chloride counteracts to a great extent the weakening effect of calcium chloride.

The addition of 6 c.c. 10 % $CaCl^2$ solution to 200 c.c. saline somewhat weakened the contractions. The addition of 6 c.c. of KCl solution had no effect, the addition of another 6 c.c. of KCl solution slightly increased the strength of the contraction, and a third addition of 3 c.c. KCl solution still more improved the strength of the contraction, but induced no other change.

I find too that potassium chloride sustains contractility and removes the effects of exhaustion. I first stimulated frequently for about 15 minutes a sartorius placed in saline solution until the contractions became weakened. The addition of 6 c.c. KCl solution strengthened the beats and sustained contractility.

In the preceding experiments we find that skeletal muscle differs from cardiac muscle in the following particulars. Contractility lasts longer in saline solution. Contractility is not improved or restored by adding lime salts to saline. Lime salts cause no prolongation of the beat, no delay in relaxation.

The contractions in saline of a muscle previously weakened by frequent contractions are strengthened by adding to saline a potash salt. A strong contraction induces much contraction remainder.

On the other hand skeletal muscle is similar to cardiac muscle in the following respects. Lime salts added to saline sustain contractility. Lime salts are antagonistic to potash salts.

We find this contraction remainder is removed or prevented by lime salts in a much less degree by sodium bicarbonate and is increased by potassium chloride.

CONCERNING EXPERIMENTS TO TEST THE INFLUENCE OF LIME, SODIUM AND POTASSIUM SALTS ON THE DEVELOPMENT OF OVA AND GROWTH OF TADPOLES. By SYDNEY RINGER, M.D., F.R.S.

A FEW years back I drew attention to the effect of calcium and potassium salts on the contraction of the frog's heart[1], and showed that the heart contractility is sustained by an adequate mixture of calcium and potassium salts in saline solution. I first employed calcium chloride and potassium chloride with bicarbonate of soda, but I subsequently found that a saturated solution of tribasic phosphate of lime[2] in ·6 °/₀ saline solution with the addition of 1 c.c. to 1·5 c.c. of 1 °/₀ solution of potassium chloride to 100 c.c. sustains normal contraction for a still longer time.

I also tested the action of distilled water and distilled water containing sodium, or potassium or calcium salts[3] on fishes. Minnows placed in distilled water die on an average in about four hours. Sodium chloride, sodium bicarbonate and potassium chloride added singly to distilled water sustain life much longer and for about the same time, about two days; sodium bicarbonate prolonging life rather longer than either of the other two salts.

Calcium chloride added to distilled water sustains life much longer than corresponding quantities of either sodium or potassium salts. The addition of sodium bicarbonate greatly enhances the life-prolonging effect of calcium chloride.

The destructive action of distilled water appeared to be chiefly due to its disintegrating action on the exposed tissues of the fish and especially of the gills.

With Dr Buxton[4] I then tested the effect of minute quantities of sodium and calcium salts on one of the simplest forms of contractile tissue, namely cilia.

We found that distilled water quickly disintegrated the tissues, separating and breaking up the ciliated cells, and so quickly arresting ciliary movements, whilst sodium and calcium salts prevent their disintegration and greatly prolong ciliary activity, sodium salts being more efficacious than calcium salts. We have here obviously an altogether different action to that by which simple saline suspends contractility of cardiac muscle. When the frog's heart is fed with simple saline contractility is lost in a few minutes, and can be restored by the addition of a calcium salt. In these experiments however, ciliary action persisted for about twenty-four hours, and the distilled water produced complete disintegration, so that contractility could not be restored by adding salts to the distilled water. Further, sodium bicarbonate cannot sustain cardiac contractility, only a lime salt can do this, whereas in the above experiments sodium bicarbonate was certainly as efficient as calcium chloride in preventing disintegration and sustaining ciliary action. With cilia it appears that distilled water affects the cement substance binding the cells together, and in a less degree disintegrates the cells themselves.

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[1] This *Journal*, Vol. IV. p. 29.

[2] This *Journal*, Vol. VII. p. 291.

[3] This *Journal*, Vol. V. p. 98.

[4] This *Journal*, Vol. VI. p. 158.

I next tested the action of distilled water and distilled water containing calcium, sodium and potassium salts on Laminaria[1]. Laminaria swells prodigiously in distilled water, and this swelling is not lessened by the addition of sodium or potassium salts, though it is greatly controlled by the addition of a minute quantity of a calcium salt. In the Fucoid Algæ the cell consists of two parts, the outer being composed of a degradation compound, namely bassorin or tragacanth, and it is this material which binds the cells together and which swells up so enormously when the dried plant is placed in water. It appeared to me probable that the cement substance which unites the animal cells together might be of a similar nature, and behave in a similar manner; and to some extent this is true, for the cement substance in both vegetable and animal tissues appears to be similarly affected by lime, thus preventing their imbibition of water, and so preventing swelling in the case of Algæ and disintegration of tissue in the case of the freshwater mussel's gills.

Recently I have continued the investigation of the action of distilled water alone or containing sodium or potassium or calcium salts on animal tissues, and shall speak first of the action on the ova of frog's spawn.

In all these experiments the fluid was renewed at the end of twenty-four hours to remove any salts that had diffused from the ova or its mucilaginous envelope.

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[1] This *Journal*, Vol. VII. p. 118.

Frog's spawn placed in distilled water does not develope at all or very little. The mucilaginous envelope swells up to four to six times the size of ova in tap water or in distilled water containing calcium or sodium salts.

In two or three days the vitelline membrane separates from the rest of the ovum and enlarges so that the ovum is seen lying in a large cell, about twice or three times its own size, and a little later between the swollen membrane and the rest of the ovum a white opaque substance forms which obscures the ovum, but is not developed equally in all parts of the sac formed of the distended vitelline membrane, so that some parts of the ovum are more obscured than others. The opaque substance under the microscope looks like a low form of organized membrane containing a few fibres.

I next tested the effect of various lime salts added to distilled water, using the chloride, the nitrate, the sulphate and the tribasic phosphate salts. With the chloride and nitrate development proceeds further than with distilled water, but not so far as with calcium sulphate, whilst this in its turn is far inferior to tribasic phosphate of lime; these results correspond to the influence of lime salts on the frog's heart, whose contractility is far better sustained by a saturated solution of tribasic phosphate of lime in ·6 °/₀ saline solution than by calcium chloride or calcium sulphate.

I used solutions of various strength of calcium chloride, adding in different experiments, respectively, 5 c.c., 10 c.c., 20 c.c. and 40 c.c. of 2 °/₀ solution to 1000 c.c. distilled water. The ova developed to a slight extent but did not usually escape from the mucilaginous envelope. They developed slowly, and did not appear to increase in size for the most part beyond the third or fourth day, but occasionally growth continued till the seventh or eighth day. The enlarged ova, as with distilled water, becomes surrounded by a sac composed apparently of the separated and enlarged vitelline membrane, and some of the opaque white material was formed between the wall of the sac and the contained ovum, though much less than in the experiments with distilled water.

In the experiments with calcium nitrate I employed 20 c.c. of 2 °/₀ solution to 1000 c.c. distilled water. The ova slowly developed and ceased to grow on the eighth to the eleventh day.

With sulphate of lime I employed a saturated solution and one part of a saturated solution to two parts of distilled water. The difference in quantity of the lime salt did not modify in any way the rate or amount of development.

For the first three or four days the ova developed in these solutions as rapidly as ova placed in tap water, afterwards they grew much more slowly so that on the seventh to the tenth day the ova were only one half the size of ova in tap water. Most escaped from the mucilaginous envelope and lay almost motionless on their side. Most died on the tenth or eleventh day, one or two living till the thirteenth day.

With a saturated solution of tribasic phosphate of lime in distilled water development progressed much better, indeed almost as rapidly as with tap water. After twenty days all were alive in the phosphate solution, but only half the size of ova placed at the same time in tap water, and they lived as long as tadpoles in tap water. In these experiments the tadpoles were of course unfed, or this would have vitiated the experiments by supplying them with other salts than the phosphates.

These experiments prove that lime salts greatly assist development of ova but not in an equal degree, phosphate of lime being far more helpful than other preparations of lime.

The amount of tribasic phosphate dissolved by distilled water is very small, only just discoverable by the oxalate of ammonium test, and it may be urged that the difference in the efficacy of the different forms of lime salts was due to the strength of the solution and not to the difference in the form of the salt.

This view is certainly not correct, for with calcium chloride the weaker solutions did not contain more lime than the phosphate of lime solution, judging roughly by the visible deposit on precipitating the lime with oxalate of ammonium.

I also tested the action of potassium chloride on the development of ova, adding 20 and 40 c.c. of 1 $^0/_0$ solution to 1000 c.c. distilled water. Very little development occurred, scarcely more than with distilled water. The mucilaginous envelope swelled a little more than the envelope of ova placed in tap water, but much less than of ova in distilled water.

It may be suggested that distilled water destroys the ova by diffusing out potassium, sodium and calcium salts which are necessary for development and growth. This explanation however fails, for in the first place salts will not diffuse from living tissues probably because they are combined with the tissues, otherwise fish would be destroyed and the tissues of the gastro-intestinal tract would constantly be destroyed after taking water. Moreover sulphate or nitrate or chloride of calcium would prevent diffusion of lime salts as completely as phosphate of lime, and indeed should do so more effectually, as in most of these experiments, chloride, sulphate and nitrate of lime solutions contained far more lime than the phosphate of lime solution.

As the ova were in most of the experiments completely covered with the solution, it may be surmised that the superiority of tap water is due to its containing more air than the various saline solutions made with distilled water. This view however fails to explain the great superiority of phosphate of lime to other lime solutions. The greater quantity of air in tap water may perhaps explain the more rapid growth of ova and tadpoles in tap water than in phosphate of lime solution, but I suggest that the more rapid development is in part due to the more abundant growth of low forms of vegetable life from the germs deposited by the dust of the room, for after many days tap water became of a slight greenish colour, and this was much more pronounced in tap water than in phosphate of lime solution.

We have seen that with distilled water and some lime solutions the vitelline membrane separates from the rest of the ovum and becomes greatly distended. Is this due to diffusion or imbibition? Not to diffusion, as phosphate of lime solution containing a very minute quantity of lime prevents this, whilst it occurs in strong solutions of chloride and of sulphate of lime.

Finally, the destructive action of distilled water cannot be due to its causing great swelling of the mucilaginous coating of the ovum, for phosphate of lime solution causes much more swelling than stronger solutions of chloride or sulphate of lime, and yet as we have seen ova in phosphate of lime solutions develope well.

I next performed a series of experiments of a similar kind with tadpoles. The tadpoles were for the most part hatched and kept unfed in tap water in the physiological laboratory. Some came from the country. Those hatched in the laboratory were about three weeks old. Whilst kept in the testing solution they were unfed[1].

Placed in distilled water tadpoles die in twelve to eighteen hours; and one sees the epithelium covering then separating in flakes showing that as in previous experiments distilled water disintegrates tissues.

Placed in distilled water containing 5 c.c., 10 c.c., 20 c.c. and 40 c.c. of 1$^{\circ}/_{0}$ solution of sodium bicarbonate in 500 of water they die in less than twenty-four hours. Hence bicarbonate of soda is unable to sustain life.

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[1] They had therefore to eat only the albuminous covering of the ova and any low organisms that grew in the exposed fluids from spores deposited from the air in the room.

Placed in a solution consisting of 5 c.c. of lime water to 1000 c.c. of distilled water tadpoles die in a few hours.

Calcium chloride in solutions containing 5 c.c., 10 c.c., 20 c.c. and 40 c.c. of 2°/₀ solution in 1000 c.c. of distilled water does not sustain tadpole life, for they died, some in six hours and the rest were found dead in twenty-four hours. The epithelium soon desquamated and was easily rubbed off.

Tadpoles placed in a saturated solution of sulphate of lime or in a saturated solution diluted with two parts of distilled water speedily die, not living more than twenty-four hours and often dying much sooner.

Carbonate of lime and tribasic phosphate of lime on the other hand sustain life for a considerable time.

The solution with carbonate was formed by mixing precipitated carbonate of lime with distilled water. Of course carbonate of lime is insoluble and if tadpoles are at once placed in this fluid they speedily die, but if the mixture is exposed several days then the fluid can sustain life, due no doubt to the formation of some bicarbonate of lime[1]. When placed in the solution exposed some days, all the tadpoles were alive after ten days but were very inactive, much more so than tadpoles kept much longer, unfed in tap water.

Tribasic phosphate of lime too is well able to sustain life. This has been already shown, for ova mature and hatch in such a solution and then continued to live from three weeks to a month. Further experiments were made. Tadpoles reared in tap water were placed in tribasic phosphate solution and lived from ten to twenty days.

In conclusion, I draw attention to the interesting fact that those lime salts most efficacious in sustaining function of cardiac tissue are those best adapted to sustain life and growth in ova and tadpoles; phosphate of lime being far more efficacious than sulphate and the sulphate somewhat more efficacious than chloride. I have not made sufficient experiments with carbonate of lime to test its influence in cardiac muscle. It would appear that those salts of lime where the lime atom is least saturated by the acid are the most capable of sustaining function.

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[1] This is shown by the precipitate that occurs when oxalate of ammonium is added to the fluid.

CONCERNING THE ACTION OF CALCIUM, POTASSIUM, AND SODIUM SALTS UPON THE EEL'S HEART AND UPON THE SKELETAL MUSCLES OF THE FROG.

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THE subject of the present paper involves many facts which have been already demonstrated as far as the batrachian heart is concerned. It will be best first to recall these facts and then to detail experiments made with a view to ascertain how the heart of the eel behaves under similar conditions, namely, when varying solutions are circulated through it.

In the case of the batrachian hearts a saline (·6 °/₀) solution fails to sustain ventricular contraction[1].

When saline circulates through the frog's ventricle the contractions rapidly get weaker; after a while a partial recovery may occur, but in about twenty minutes the heart ceases its spontaneous beats and is inexcitable by even strong induction shocks. When the contraction is weakened dilatation of the ventricle is delayed, and later on the same delay reveals itself in the systole. In the same series of experiments it was shewn that in physiological doses potassium salts added to the circulating saline do not enable it to maintain the contractility of the ventricle, indeed under their influence the ventricle ceases to contract sooner than when simply saline is circulated.

It was also demonstrated that bicarbonate of sodium in saline cannot support the heart's contractility. This salt has the power of restoring contractions in some degree when these have stopped, owing to the ventricle being fed with saline; but after some minutes, subsequent to the addition of the soda salt, the beats again cease and the ventricle stops in partial systole.

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[1] On the Influence of the Blood Constituents on the contraction of the Heart. S. Ringer, this *Journal*, Vol. IV. No. I. p. 33.

In the case of lime salts the following facts were elicited. Calcium chloride added to saline, when the heart has its beat greatly weakened or arrested, will initiate vigorous beats which however rapidly take on a character peculiar to lime salts, and differing widely from the trace obtained when blood solution is used. Thus, the trace grows broader, the summit becomes rounded, and dilatation is greatly delayed. It was also shewn that these effects of lime can be completely obviated by adding a physiological dose of potassium chloride. Thus, although potassium chloride in saline is less capable of promoting contraction than saline by itself, and while calcium chloride in saline produces great delay in the dilatation of the ventricle with fusion of the beats, the mixture of these salts produces a normal beat. While however potassium chloride does away with the broadening of the trace due to the lime, it also lessens the height of the trace, or in other ways somewhat weakens contraction while it hurries dilatation. Now the addition of a physiological dose of bicarbonate of sodium to this fluid obviates the weakening effect of the potassium salt and induces complete contractions. And this fluid, consisting of saline containing physiological quantities of calcium chloride, potassium chloride and sodium bicarbonate, will sustain good contractions for a considerable time. However, subsequent research demonstrated that a circulating fluid so composed did not sustain the heart's contractions equally well for all seasons of the year.

And we found that a circulating fluid consisting of saline saturated with calcium phosphate, with a physiological quantity of potassium chloride, was much more efficacious in maintaining normal contractions, which continued for several hours and were not affected by the season of the year.

It was determined to test whether the results arrived at above would hold good for the fish's heart. The common eel (*Muraena anguilla*) was selected and was experimented upon in a Roy's tonometer. The eel's heart consists of a venous sinus and auricle and a ventricle; it is fixed in its pericardial chamber by fibrous bands, in some of which vessels run to supply the heart. From the ventricle a good sized aorta springs; this vessel is bulged out just as it leaves the ventricle.

The tissue of the eel's heart is extremely friable and hence manipulation of the ventricle itself is almost impossible, while the structure of the sinus and auricle render them unsuited for the introduction of a cannula. As to the nourishment of the heart muscle, it is as stated above supplied in part by vessels running in the pericardial bands, but is in the main fed by imbibition, the inner part of its substance being spongy.

With respect to the method of our experiments we may say that after some initial trials it was found best to open the aorta, having previously ligatured the heart in the auriculo-ventricular groove. The aortic valves are then cut by scissors introduced into the aorta and the perfusion cannula passed into the ventricle through the aorta, care being taken that it really travels well beyond the valves. The cannula is then tied in and the other parts cut away from the ventricle. The heart may then be placed in the oil chamber, and will beat vigorously for very many hours provided the circulating fluid be favourable; indeed the eel's heart will outlive a frog's heart. The trace obtained from the heart of an eel of the weight of rather less than a pound is excellent. It resembles the trace obtained with the frog's heart; although somewhat larger, its details are much the same, so that the descriptions of the traces are quite comparable.

When the eel's heart, prepared as above described, is fed with a blood solution it beats vigorously; as soon however as this is replaced by a saline solution (·6 $^0/_0$) the beats rapidly grow weak, and in three or four minutes almost cease. If now potassium chloride solution (1 $^0/_0$) be added in proportion one c.c. to the hundred, no return of the contractions occurs. So that the eel's heart, like that of the frog, cannot contract either with saline or saline and potassium chloride circulating through it. Bicarbonate of sodium, added directly to a saline fluid, was quite inadequate to support the ventricle. At first the usual saline diminution of the beat took place; the bicarbonate was added, 2 c.c. of 1 $^0/_0$ solution to each 100 c.c.; the weakening continued for some time, when a faint increase in the size of the contraction appeared, but this did not last. In this respect the result quite agrees with what obtains in the case of the frog's heart. The addition of potassium chloride to such a solution does not revive or maintain the beat; although a barely appreciable increased amplitude in the trace follows, this soon wears off; so that in respect of this slight improvement the eel's heart differs from the frog's heart.

We next experimented with lime salts. When an eel's ventricle, beating well with a blood circulating fluid, had that medium replaced by saline and the contractions had become very feeble, the saline was replaced by saline saturated with phosphate of lime. The contractions speedily increased in strength until they became complete; there was however great delay in dilatation, leading to fusion of the beats.

When the ventricle was left exposed to this circulating fluid the contractions weakened, persistent spasm appeared, and with it still more considerable delay in dilatation.

In all these particulars a striking correspondence is seen to exist between the eel's heart and that of the frog. And in working with the eel's heart we further found, that it was necessary to add the phosphate of lime very soon after the heart had been weakened by saline, otherwise little or no amendment followed the addition of this lime salt. If however such failure to effect recovery did occur, we found that adding potassium chloride in adequate quantities always resulted in complete recovery. The actual dose of potassium chloride varied, although 3 c.c. of 1 $^0/_0$ solution in the 100 c.c. phosphate saline was the best working quantity we obtained.

The delay in dilatation which followed supplying a vigorous ventricle with phosphate of lime saline was however obviated by half this dose of potassium chloride.

Further, our observations show that, as in the case of the frog's heart, the heart of the eel can be sustained for several hours, giving good beats when supplied with phosphate of lime saline to every hundred c.c. of which 1 c.c. potassium chloride 1 $^0/_0$ solution had been added.

But we also found that after several hours the beats, although maintaining their normal character, became much weakened, and then the addition of further quantities of chloride of potassium were able to restore the contractions completely. Subsequently a further decline of the contraction was again obviated by adding more potassium chloride, and this was repeated again and again until in some cases we kept the heart contracting completely with as much as six c.c. of the potassium chloride solution in the hundred of phosphate of calcium saline.

We next investigated the action of calcium chloride.

The eel's ventricle was first started with a blood circulating fluid, and this was shortly changed for a simple saline fluid, and so soon as the heart's beats had almost stopped 1 c.c. calcium chloride (one per cent. solution) was added to each 100 c.c. The beats soon grew stronger, but persistent spasm, with delay in dilatation, came on almost immediately together with a certain degree of fusion. So marked were these "lime effects" that subsequent addition of even large doses of potassium chloride solution altogether failed to counteract them.

Summarising the results we have arrived at, experimenting with the eel's heart, we may say that, like the frog's heart, it cannot maintain its contractions when supplied with a circulating fluid composed of (*a*) simple saline, or (*b*) saline plus potassium chloride, or (*c*) saline plus sodium bicarbonate, or (*d*) saline plus both these salts.

(*e*) Phosphate of calcium added to saturation to saline can sustain contractility but leads to great delay of dilatation with persistent spasm and fusion of the beats.

(*f*) This delay of dilatation, this persistent spasm and fusion of the beats, are completely obviated by the addition of potassium chloride.

(*g*) So that we find we possess an excellent circulating fluid capable of maintaining the heart for several hours, which consists of phosphate of calcium saline, with potassium chloride.

(*h*) That calcium chloride solution is inferior to the phosphate in its powers of supporting contractility.

(*i*) So that we find from the preceding statement of our experiments hat the frog's and eel's heart correspond in almost all particulars.

[*From the Journal of Physiology. Vol. VIII. No. 5.*]

UPON THE SIMILARITY AND DISSIMILARITY OF THE BEHAVIOUR OF CARDIAC AND SKELETAL MUSCLE WHEN BROUGHT INTO RELATION WITH SOLUTIONS CONTAINING SODIUM, CALCIUM AND POTASSIUM SALTS.

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THE experiments narrated in the present paper were undertaken with the view of testing the action of physiological doses of the salts of calcium, sodium and potassium upon skeletal muscle and to compare such effect with that exercised by the same substances upon cardiac muscle.

With regard to the heart we have already shown the following facts to be true. When a simple saline solution (·6 $^0/_0$) is circulated through the detached heart of the frog, the cardiac contractions rapidly grow weaker and in about 20 minutes[1] contractility ceases. As the contractions lessen in amplitude, diastolic dilatation becomes slightly delayed, and later a similar slight retardation in systolic contraction occurs. Also, when a salt of lime (the chloride or tribasic phosphate or other lime salt) is added to this saline, it enables the ventricle to contract, producing a trace with a rounded top and marked broadening, while diastolic dilatation is considerably delayed. When potassium chloride was added in a physiological dose to the circulating saline, it failed to sustain contraction, the heart rapidly coming to a standstill in diastole. But when potassium chloride was introduced into a circulating fluid consisting of saline and phosphate or chloride of calcium, it antagonised, so far as to obviate the broadening, pointing the trace and slightly lessening its height while it abolished all delay in dilatation. Such a fluid will maintain normal beats for several hours provided the circulation be kept up. These phenomena we have shown to obtain alike for the heart of the frog, of the eel[2] and of the tortoise[3]. In explanation of the above effects we have demonstrated that, while in the case of the frog the fluid must act directly by imbibition from the ventricular cavity, so in that of the eel and tortoise the method adopted effectually prevented the coronary circulation coming into play; hence, in each instance, the heart was fed by imbibition from the fluid contained in its cavity. We further indicated that the intervention of the nervous system is probably absent, since when a heart is inhibited through the vagus its muscular tissue is still fully susceptible to faradic stimulation, whereas when the ventricle is stayed by the perfusion of saline, or potassium chloride, the muscle no longer responds to strong shocks, although it recovers its excitability when a suitable fluid (i.e. one containing lime) is circulated through it.

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[1] This *Journal*, IV. 1.

[2] This *Journal*, VIII. 1.

[3] *Medical Record*. New York, April 30, 1887.

We find, as stated in a preceding paper[1], that solutions of the salts in question need to exert a certain pressure on the interior of the ventricle in order that they may influence the heart muscle in the manner above mentioned. For, if the heart of a tortoise be immersed in saline, the aorta being attached by a pin to a loaded cork with a thread tied around the frenum which exists at its apex and secured to a recording lever, we found that although the contractions grew successively weaker they did so very slowly indeed. At the end of an hour and a half they were reduced to only a moiety of their initial size. We then removed the saline, replacing it by phosphate of calcium in saline, but failed to restore strength to the contractions, and additions of potassium chloride, bicarbonate of sodium were attended by similar failures. A subsequent attempt to restore normal contractions by perfusing phosphate of calcium saline circulating fluid (i.e. saline saturated with tribasic phosphate of calcium with 1 °/₀ of a 1 °/₀ solution of chloride of potassium) was unsuccessful.

A difference thus marked in the behaviour of the heart muscle suggested that possibly facilities for diffusion, osmosis and filtration between the endocardial and pericardial surfaces might be in favour of the endocardium. This hypothesis seemed to be affirmed by an experiment in which we placed the heart of a tortoise, after longitudinal bisection, in saline solution, as in the case of the whole heart above mentioned. The initial beats were very strong but soon became weakened. After fifty minutes the saline was changed for phosphate of calcium circulating fluid, and as a result the contractions rapidly recovered their primary strength. A return to saline was followed by diminution in strength of beat, which was only in part obviated by substituting saturated solution of tribasic phosphate of calcium in saline solution containing 2 °/₀ of a 1 °/₀ solution of potassium chloride.

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[1] *Medical Record.* New York, No. 860, p. 482.

We now pass on to the consideration of skeletal muscles, with which we have already experimented by immersing them in solutions of salts[1], arriving at the following results. The detached gastrocnemius of a frog was placed in the test fluid, which was changed thrice daily, and the muscle was tested with the faradic current. We found solutions of calcium salts in saline enabled the muscles to maintain contractility longer than saline plus sodium bicarbonate or potassium chloride. The line of experiment was subsequently varied in the following way. We placed the sartorius in the test solution after carefully severing its pelvic attachment and cutting off the bones above and below the knee. Contractions were frequently excited, the electrodes being applied to the lower end of the muscle, and graphic records were taken, and care was exercised that the muscle should be simply suspended and not stretched. In this way we tested the effects upon the immersed muscles produced by saline solution, lime and potash salts.

In a ·6 °/₀ saline solution contractility persisted from an hour and a half to two hours; whereas the heart, perfused with a similar fluid, ceases to be contractile in a few minutes, while marked contraction remainder (contracture) appears if the rapidity of the excitation be not too great. The addition of a lime salt does not restore or improve contractility, nor does it cause a prolongation of the beat or delay in relaxation. The addition of potassium chloride to the saline is found to strengthen contractions previously weakened by frequent excitations. In all these respects skeletal muscle contrasts with the results arrived at in experiments made upon perfused cardiac muscle.

We found however that skeletal muscle agrees with cardiac muscle in the following points.—

Lime salts, when added to the saline in which the muscle is suspended, are capable of sustaining contractility and are further antagonised by potash salts.

In the present research a different method was adopted, the test fluid being circulated under pressure through the capillaries of the skeletal muscle. In this way the skeletal muscle was placed under similar conditions to the heart when perfused by test fluids through the ventricular cavity, as in the preceding experiments. We first applied this method to the tortoise[1], inserting a cannula into the descending aorta, permitting an outflow through another cannula inserted into the abdominal vein. Saline circulated in this way took some time to remove all the blood from the vessels; as soon, however, as this was accomplished the excitability to the faradic current rapidly diminished and had almost ceased in an hour and three-quarters. Upon replacing saline by phosphate of calcium saline[2] a return of contractility took place.

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[1] This *Journal*, VII. 4.

We then returned to saline, and again contractility disappeared, to be restored by returning to the phosphate of calcium saline; and this manœuvre was effected several times, always with the same result.

Subsequently this subject was pursued by the employment of frogs instead of tortoises. A fine cannula was inserted and securely tied into the aorta just as that vessel leaves the ventricle, and the heart cut away so as to allow a free outflow of fluid from the venous sinus. The frog was placed upon its back and a free circulation maintained through, by raising the feeding vessel several inches above the frog board. The outflow of fluid was led away from the part experimented upon so as to avoid the possible fallacy likely to arise if the part were bathed by the circulating fluid after it had traversed the vascular system. Two series of experiments were prosecuted, in one of which the muscles of the tongue were employed and in the other gastrocnemius. When the tongue was used, we secured the head, by a pin passed through the skull and carefully placed away from any large vessels; the tip of the tongue was then tied out of the mouth by thread to a recording lever. Electrodes were inserted, one (a pin) at the base and one (a slender hook) at the tip. The lever was capable of being weighted, and indeed was so weighted in some experiments, while it carried a writing pen at its end for tracing the muscle curve contractions upon a blackened revolving cylinder.

In the second series when we made use of the gastrocnemius we fixed the frog by its head, made a small skin incision over the tendon of the gastrocnemius, which we divided and securely tied by a thread to the lever. Fixed in this way the muscle travelled easily and was yet kept moist and maintained in free vascular communication with the general system. The electrodes were fixed one (the pin) in the knee and the other (the hook) in the tendinous end of the muscle. The lever was weighted.

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[1] *Medical Record.* New York. April 30, 1887.

[2] Saline solution saturated with tribasic phosphate of calcium.

In every case it was easy to determine whether a thorough circulation was effected, for as soon as saline entered the extremities quivering took place and marked œdema subsequently supervened.

When a saline solution (·6 $^0/_0$) was perfused in the manner above described, so soon as the blood was washed out of the vessels the muscles of the tongue and extremities and, in some cases even those of the trunk, showed marked fibrillary contractions. On stimulation the whole muscle so excited contracted, but these contractions gradually grew weaker, ceasing in some instances after a few hours and persisting in others for twenty-four, though very much weakened. This took place even when the circulation through the muscles was found to be rapid.

But when saline solution is perfused, besides the weakening which the contractions undergo, a difference in the form of the muscle curve is observed. While the rate of contraction is slowed the period of relaxation is often enormously prolonged; and these changes are constant until contractility has quite disappeared. In some experiments we found that soon after the commencement of perfusion the amount of contraction, as shewn by the height of the trace, is greatly increased, although the strength of the excitation remained the same. Comparing numerous experiments we found that the length of time contractility persisted and the amount of delay in the period of relaxation to vary very considerably, and we believe this variation to be largely dependent upon the condition of the frog.

The perfusion of saline solution, then, affects the skeletal muscles in much the same manner as does immersion in saline solution. Fibrillary contractions occur in both cases, and also marked delay in relaxation.

Compared with results obtained by perfusing saline solution through the ventricle, perfusion through skeletal muscle contracts; for contractility in the last case persists much longer, relaxation is very much more delayed, while dilatation of the ventricle, as contraction grows weaker, is very little retarded.

We are then led to enquire, are these differences betwixt cardiac and skeletal muscle due to a diversity in the molecule of the muscular tissue or to the different way in which the protoplasm of the contractile tissue is built up? We will in the first place restrict our remarks to the variation in the time during which contractility persists in these two kinds of muscular tissue. Skeletal muscles are very plentifully supplied with capillaries, and this circumstance might be supposed to indicate that they would allow their nutritive material and salts to be washed out more readily than the trabecular structure of the ventricle would permit. The following considerations will however explain that such is probably not actually the case. The trabeculæ are, as a matter of fact, minutely subdivided up until the divisions are of microscopic size and the whole surface of these is bathed with the circulating fluid. Further, as the heart muscle has no sarcolemma, we think that salts and albumin would diffuse out more quickly from the trabecular structure than from that of skeletal muscle covered as each fibre is by its sarcolemma. Again, even the abundant surface for diffusion &c. offered by the capillary network in skeletal muscle does not equal that presented by trabeculæ, the most minute subdivisions of which are bathed with the circulating fluid. The sarcolemma covering the skeletal muscles would presumably be quite sufficient to prevent diffusion of the colloidal matters, such as albumin &c. and this retention of albumin in the muscle would suffice to sustain contractility for a long time, for we have shown that serum when added to saline solution in even minute quantities has power to sustain ventricular contractility for a considerable time. The same holds good for dialysed serum, cardiac contractions being kept up for a lengthened period when the heart is fed with saline plus the dialysed serum.

But although the contractility of cardiac muscle is thus maintained, the character of the contraction is, as we have pointed out elsewhere, materially altered, dilatation being much delayed; a trace of the muscle curve taken under these circumstances resembles very exactly the trace obtained from a heart fed with saline solution containing a lime salt. We find further that if the dialysed serum be incinerated the ash still contains lime salts, so that dialysis fails to remove the lime which appears in combination with the molecule of albumin. Hence we are unable to say whether contractility of the ventricle is sustained by the serum or by the lime which is associated with it. It is however noticeable that the tracing which results from saline and a lime salt, like that obtained from saline and dialysed serum, is affected by a physiological quantity of a potassium salt, and in both instances the dilatation is accelerated and a normal trace produced.

Although we are not able to say whether the influence of serum is due to the albumin it contains, or to the lime associated with it, the answer to this question does not really affect the matter which is under our immediate notice; we think that the sarcolemma by lessening diffusion checks the escape of albumin from the skeletal muscle, and by this we should explain how it is that, when perfused with saline, skeletal muscle retains its contractility so much longer than does cardiac muscle when similarly treated.

Now further, the muscle curve resulting from the contraction of skeletal muscle perfused with saline differs in form from that of cardiac muscle similarly treated. Relaxation in the case of skeletal muscle is very greatly retarded, while this is only slightly the case with cardiac muscle. This alteration in the curve of skeletal muscle resembles closely the trace derived from muscle exhausted by repeated excitations. The rate of contraction is somewhat slowed, while that of relaxation is enormously retarded. Indeed while the line of ascent is rendered more oblique, that of descent is made very much more so when compared with the normal tracing. In some experiments changes other and in addition to those described above were found to develop after a time; thus in from ten minutes to half an hour after perfusion the amount of contraction following each induction shock was much increased, so that the curve rose considerably above the base line. This last phenomenon, however, we did not find to be constant. The curve described immediately above resembles that obtained by the perfusion of veratria in solution.

Perfusion of saline through the muscle produces, as indeed one would expect, exhaustion effects, sometimes giving rise to "irritable weakness," that is, the excitations for a time produce a greater amount of contraction together with a retardation in relaxation. On the other hand, as is well known, cardiac muscle does not show fatigue effects like skeletal, and this would suggest that the protoplasm of these two varieties of muscle differs somewhat in its composition.

We next made experiments in order to test what effect the introduction of a lime salt in solution would have upon the curve derived from skeletal muscle weakened by perfusion of saline solution. We employed a saturated solution of phosphate of lime in saline to every hundred c.c. of which one c.c. of a one per cent. solution of chloride of calcium was added. In many experiments this addition strengthened the contraction, making the curve higher and accelerating both contraction and relaxation. These results in some respects correspond, but in others differ from what obtains when lime salts act on cardiac muscle, for in this case acceleration occurs while the amount of contraction is increased. But unlike skeletal muscle relaxation (dilatation) is delayed by a lime salt. This apparent contradiction can, we think, be readily explained, for it must be remembered that cardiac differs from skeletal muscle, as we have pointed out, in that, no matter how far exhaustion be carried, it does not produce delay of relaxation (dilatation) whereas exhaustion in skeletal muscle at once causes delay in relaxation. And, as we have above seen, saline solutions, by exhaustion, cause great delay in relaxation of skeletal muscle. Since, however, the addition of a lime salt improves the condition of the muscle and enables it to contract better, contraction and relaxation are accelerated while the amount of contraction is actually increased.

The extent to which recovery occurred after the addition of a lime salt varied greatly in different experiments. We found that where the effect of saline showed itself soon, and the lime salt was thrown into the circulation early and before much œdema had developed, recovery was marked; where however œdema was extensive, recovery was delayed—often not appearing till after the lapse of many hours. In such cases the slow and slight improvement was doubtless accounted for by the œdema. For, in œdematous tissues the circulation becomes more and more obstructed, hence, lime salts reach the capillaries more slowly, these being almost closed by pressure.

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REGARDING THE ACTION OF LIME SALTS ON CASEINE AND ON MILK.

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IN the following experiments I prepared caseine by adding commercial rennet[1] neutral in reaction to milk, and left the mixture in a warm bath of about 40° C. for three to five hours. I obtain the reactions detailed in this paper, best with caseine produced by adding 20 c.c. of rennet to 700 of milk.

Lime water freely dissolves caseine. To ensure the water being saturated with lime, I kept the bottle containing the lime and distilled water in an ice chamber. I well rubbed the caseine with the lime water in a mortar, and placed the flask containing the mixture in a cool room near an open window, or if the weather was warm, in an ice chamber. In all these experiments I used 10 c.c. of the solution of caseine.

The following experiment is a good example of the behaviour of this solution of caseine on the addition of calcium chloride[2] with the aid of heat.

I mixed caseine, prepared four days previously, with lime water and after standing twenty-four hours I filtered it. To 10 c.c. of the clear filtered caseine solution I added one drop of the calcium chloride solution, without producing any apparent change in the fluid. I then placed the test tube in a bath of 70° C., the fluid rapidly became milky, first at the top and then the milkiness extended through the whole fluid. On placing the test tube in iced water the fluid rapidly became quite clear again. I then added more calcium chloride solution in all four drops, without changing the appearance of the fluid and again placed the test tube in warm water at 70° C. and the solution quickly clotted; on replacing the test tube in iced water, the clot dissolved and the milkiness quite disappeared. I then increased the quantity of calcium chloride to six drops, the fluid becoming a little milky whilst cold. On placing the test tube in water at 70° C. the solution clotted and I was unable to completely dissolve the clot by the aid of ice water. The clotting of the solution by heat and its solution and the removal of all milkiness by the aid of cold can be repeated many times. If a still larger quantity of calcium chloride as nine to ten drops is added to the 10 c.c. of the caseine solution, the fluid clots, or curds separate at the temperature of the room.

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[1] Prepared by Crosse and Blackwell, and contains a considerable quantity of common salt and some lime salts.

[2] I always used a 10 % solution of calcium chloride. Calcium chloride dissolved in distilled water gives a strong alkaline reaction, I neutralized this alkalinity by adding a weak solution of hydrochloric acid till the solution became faintly alkaline.

The clot formed by the action of calcium chloride, has all the appearance of a clot produced in milk by rennet and if allowed to stand, in a short time it contracts and the rapidity and amount of contraction are greatly aided by heat, the clot whilst shrinking, becoming firmer. This is well shown when sufficient calcium chloride is added to clot a cold solution. On placing the test tube in a bath between 60° and 70° C. the clot rapidly shrinks.

If the curd deposited by calcium chloride solution and heat is heated to 80 or 90° C., then the milkiness or clot is not dissolved by cold. This is well shown by heating a saturated solution of caseine in lime water to 80 or 90°. The fluid becomes milky and in placing the test tube in ice, the cloudiness does not disappear.

We see from the foregoing experiments that the addition of calcium chloride[1] produces a compound of caseine, that is more soluble in cold than in hot solutions. If the compound formed is small in quantity, as happens when but little caseine is dissolved by lime water, or when a smaller quantity of calcium chloride is added to a stronger solution of caseine, the compound is quite soluble in iced water, and only slightly insoluble at a temperature of 40° or 70°, and hence the fluid becomes only milky. If more of the compound is produced, it is still soluble at a low temperature, but at 40°, or higher say 70°, it clots and both the milkiness and clot disappear on again cooling the fluid. If a larger quantity of the compound is produced, the fluid becomes cloudy at a low temperature, and clots at a higher, and then, on cooling the solution some of the clot remains undissolved. On adding still more calcium chloride, a clot forms even at the temperature of iced water, but a high temperature greatly accelerates the clotting and the subsequent contraction and increases the amount of contraction. At a high temperature as above 70°, if this is at all prolonged, the compound is changed in this respect, that it will not again dissolve even at 0° C.

In some instances heat merely accelerates the action of calcium chloride, for if allowed to stand some hours at the temperature of the room, the calcium chloride curds or clots the solution, but clotting at once occurs at a temperature between 40° and 60° C. In other instances heat is necessary to enable the calcium chloride to precipitate the curd.

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[1] Calcium nitrate is also efficient.

As many lime salts are more soluble at a low than a high temperature, it is probable I think that caseine combines with lime and behaves as a lime salt.

As the above reactions follow the use of calcium nitrate as well as calcium chloride, it appears that any highly soluble lime salt will similarly affect caseine.

These experiments suggest that in clotting by rennet, two processes occur. The rennet changes the caseinogen to caseine and then this combines with lime and is precipitated especially by heat, the lime compound being less soluble in warm solutions, and we shall see subsequently if too little lime is present rennet may change caseinogen into caseine, but no clotting or curdling occurs till a lime salt is added. The clot is merely a precipitate and can be redissolved and reprecipitated at will.

There appear however to be different kinds of caseine, as the preparations I obtained varied greatly as regards their solubility in lime water. Moreover caseine changes by keeping in respect of its behaviour with calcium chloride. One specimen was quite insoluble in lime water or in a weak solution of caustic soda, the day after its preparation, but on subsequent days was freely soluble. Specimens after solution in lime water, when kept a few days, often become much more readily precipitated by calcium chloride and heat. For instance we get solutions which are only slightly curded by calcium chloride, but on straining and adding a ten per cent. solution of acetic acid an abundant deposit occurs showing that the dissolved caseine has been only partially precipitated by the lime salt. If kept a day or two longer, much more curd is deposited by calcium chloride. Caseine too by keeping many days at last becomes less and less soluble in lime water. No doubt in all specimens there is a mixture of caseines, or rather modifications of caseine; as the following experiment shows.

The caseine was prepared by adding 15 c.c. of rennet to 700 c.c. of milk, the mixture being kept in a warm bath of 38° C. for four hours. This was moderately soluble in lime water but a good deal was left. To the residue I added fresh lime water but none was dissolved although the lime water and the caseine remained mixed for three days, for on filtering, no curd was deposited neither by calcium chloride and heat nor by the addition of a ten per cent. solution of acetic acid.

That caseine changes is further shown by the following fact. If a solution of caseine is exposed to the air for some days, it becomes very milky first on the surface and then the milkiness extends throughout the fluid, and after a while abundance of curd is deposited. On the other hand caseine allowed to remain in distilled water many days till it begins to be putrid will dissolve in lime water but little or none is deposited by calcium chloride and heat, and on standing exposed to the air it does not become milky, nor does it deposit curd.

As in those specimens made cloudy by exposure the milkiness begins first at the surface of the fluid it is probable that the change in the caseine is due to oxidation[1].

As lime water dissolves whilst chloride and nitrate precipitate caseine, it would appear that they act antagonistically, and this is true to some extent, but with the solution in lime water more is dissolved at a low than a high temperature. For if a saturated solution of caseine in lime water is heated to 70° C., the fluid becomes a little milky first at the top[2] and then the milkiness extends throughout the fluid, but the milkiness is only slight. The amount of lime in lime water is too small to precipitate much curd at 70° C. but on the addition of a drop or two drops of calcium chloride 10 °/₀ the precipitation of curd is much increased, so that in many cases the fluid clots. In virtue of what property does lime water dissolve caseine? I think by means of its alkalinity, for other alkaline solutions as ·2 °/₀ solution of caustic soda, or of caustic potash, also baryta water freely dissolve caseine and on the addition of calcium chloride solution especially aided by heat the solutions behave as lime water solutions, but differ in degree, the reactions being less with the caustic soda and caustic potash solutions, whilst the baryta water solutions give but little deposit on the addition of calcium chloride, even when assisted by heat.

I next proceeded to learn how the salts contained in milk affect the clotting of caseine by calcium chloride and heat.

On April 12 I prepared some caseine by adding 20 c.c. of commercial rennet to 700 c.c. of milk and placed the beaker in a warm bath of 35°. In five hours and a half I poured off the whey, pressed the caseine in muslin and washed it; I then rubbed some with lime water (which had been kept in a cool room) in a mortar and let the mixture stand 19 hours; I then filtered a part, and found it gave an abundant precipitate on adding acetic acid, proving that the caseine was freely dissolved in the lime water. To 10 c.c. I added three drops of calcium chloride solution, without making the cold fluid turbid, on heating it became turbid at the top at 35° and at 45° milky throughout. The turbidity quickly cleared on placing the test tube in cold water; I then added ·5 c.c. of 10 % solution of sodium chloride about the quantity of salts in milk, and again heated; at 35° the fluid became opaque at the top and milky and curdy at 40°. This turbidity rapidly cleared on placing the tube in cold water. I then added ·5 grammes of sugar of milk. It became cloudy at 20° and set into a jelly at 30°; and this jelly dissolved completely and the milkiness disappeared, on placing the tube in cold water.

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[1] I proved that the cloudiness is not due to a carbonate of lime being formed.

[2] The appearance of the milkiness at the top of the fluid no doubt is due to the warmer fluid rising, so that the upper part of the fluid is the highest in temperature.

In this experiment sodium chloride had no effect on the precipitation of caseine, whilst sugar of milk greatly favoured the precipitation.

Next morning I filtered the rest of the solution of caseine and made the following experiment.

Into four test tubes I measured 10 c.c. of the solution and to each added three drops of calcium chloride solution. To the fluid in the second tube I added ·5 c.c. and to the third tube 1 c.c. of 10 % solution of sodium chloride. To the fourth tube I added ·5 grammes of sugar of milk, on heating the first three became cloudy at the top at 38° and very milky at 45°, whilst the fluid containing the sugar of milk became milky at 20° and set into a jelly at 30°.

The fluid filtered very slowly, hence I was compelled to wait till next day before I obtained enough for the following experiment, on April 15th. As before I measured 10 c.c. into four test tubes. To the second I added one drop of calcium chloride solution. To the third also one drop of calcium chloride solution and 2 c.c. of sodium chloride 10 % solution. To the fourth also one drop of calcium chloride solution and ·5 gramme of sugar of milk. The fluid in the last tube even before being heated became a little opaque, at 20° C. this opacity increased, at 35° it looked like milk and at 45° set into a jelly. The second and third tubes became turbid at 35° and milky at 45°. The first tube containing caseine only dissolved in lime was a trifle opaque at 55° and became like largely diluted milk at 70°. Placed in cold water the fluid in the first and fourth tubes dissolved most; indeed, they became as they were before being heated. The fluid of the other two tubes, though clearing considerably, remained a little thick.

These experiments, which I have repeated, prove that sodium chloride in ·5 and 1 % and 2 % solutions, does not modify in either direction the effect of calcium chloride in precipitating caseine: whilst sugar of milk greatly aids the action of the lime salt.

We shall subsequently see that in these respects caseine dissolved in lime water contrasts strikingly with milk, for with milk sodium chloride antagonizes the action of calcium chloride, and a higher temperature is required to precipitate curd, whilst sugar of milk does not modify the effect of calcium chloride.

I also tested the effect of magnesium sulphate on clotting by calcium chloride and find magnesium sulphate greatly assists calcium chloride in precipitating caseine, as the following experiment shows.

To 10 c.c. of caseine dissolved in lime water I added three drops of calcium chloride solution. To another 10 c.c. also three drops of calcium chloride and ·5 c.c. of 10°/₀ solution of sulphate of magnesium. The first became curdy at 60°, whilst the fluid containing sulphate of magnesium became curdy at 32° and a much larger quantity of caseine was deposited than in the tube without magnesium sulphate.

I find moreover that sulphate of magnesium without the help of calcium chloride will precipitate caseine.

To 10 c.c. caseine dissolved in lime water I added ·5 c.c. of magnesium sulphate 10 °/₀ solution and the caseine clotted at 30° C.

To another 10 c.c. I added 1 c.c. magnesium sulphate solution and this mixture clotted at 20° C., with 2 c.c. magnesium sulphate curdled at 17° C.

We shall find when treating of the action of magnesium chloride on milk that it affects milk in quite an opposite way, antagonizing to some extent the action of calcium chloride.

The action of Calcium Chloride, alone or with other Salts on Milk.

I next tried the action of calcium chloride on milk. Large doses of calcium chloride at the temperature of the room, i.e. 10 to 15° C. cause no curdling of milk, in this respect differing strikingly from solution of caseine in lime water, from which one to three drops of calcium chloride solution precipitates abundance of curd or sets the fluid into a jelly.

Calcium chloride, however, abundantly precipitates curd from milk with the assistance of heat. The smaller the quantity of calcium chloride the higher the temperature required.

To ten c.c. of milk I added respectively 15, 10, 8, 5, 4, 3, 2, and 1 drops of calcium chloride solution and placing the test tubes in a bath, slowly raised the temperature. The tubes containing 15 and 10 and 8 drops curdled at 52°C. and became solid at 59°C. The fluid containing five drops became curdy at 55 and clotted at 58. The fluid with four drops clotted at 65, whilst the milk containing three drops became rather curdy at 68 to 70 and almost solid at 70 to 75. The tube with two drops became a little curdy at 80, and very curdy at 81. The temperature rose to 90, but did not curdle the milk with only one drop of calcium chloride solution.

Numerous experiments show that three drops of calcium chloride solution to 10 c.c. of milk, curdles the milk, setting it into a jelly at a temperature between 70 and 75° C., and that one drop of calcium chloride solution to 10 c.c. of milk is insufficient to clot milk even at boiling point.

Milk then with calcium chloride behaves like caseine dissolved in lime water, but milk requires a much higher temperature, to enable calcium chloride to precipitate curd, than caseine solution.

All these specimens gave a faint acid reaction to test paper though they smelt and tasted quite sweet. On neutralizing the acidity with either a solution of caustic soda or sodium bicarbonate, 10 c.c. milk with three drops of calcium chloride solution did not become curdy even on raising the temperature above 80° C., but on adding another three drops of calcium chloride solution an abundant curdy deposit ensued.

The acid state then decidedly favours the action of calcium chloride on milk, but is not essential, a larger quantity strongly curding milk alkaline in reaction.

Is it that the acid state favours; or the addition of soda hydrate or bicarbonate antagonizes the calcium chloride? We shall subsequently see that a small quantity of a sodium or potassium salt greatly antagonizes the action of lime chloride, but the acid state appears to favour the action of calcium chloride, for a few specimens of milk gave a faint alkaline reaction and these required a temperature of 5 to 10° C. higher, than milk faintly acid, to curdle with calcium chloride.

The curd, deposited by a small quantity of calcium chloride (as two drops) at a high temperature of 80 to 90°, on cooling in an ice chamber dissolves in parts but not completely.

As chloride of calcium solution does not clot milk first boiled and then cooled, it is evident that the high temperature does not alter the caseinogen and thus enable lime chloride to precipitate it, but that the high temperature is necessary to enable lime chloride to precipitate (combine with) caseinogen.

Salts like sodium chloride and potassium chloride oppose the clotting of milk by calcium chloride assisted by heat (in this respect clotting by calcium chloride corresponds to clotting with rennet). For instance 1 c.c. of 10 $^{0}/_{0}$ solution of sodium chloride to 10 c.c. of milk requires from 10 to 14° C. higher temperature than the same quantity of milk to which 1 c.c. of distilled water is added. I record one experiment illustrating this antagonism.

I took four specimens of milk very faintly alkaline of 10 c.c. each. To the first I added 1 c.c. of distilled water, to the second 2 c.c. of distilled water, these served as controls. To the third test tube I added 1 c.c. of 10 $^{0}/_{0}$ of sodium chloride and to the fourth test tube 2 c.c. of sodium chloride solution. I added six drops of calcium chloride solution to each test tube.

The first two specimens, those containing the distilled water clotted at a temperature between 65 and 70°. The third, containing 1 c.c. of sodium chloride, clotted at 88, and the milk in the fourth test tube containing 2 c.c. of sodium chloride solution clotted at 92.

On the following morning, from the same sample of milk which still gave a faint alkaline reaction I tested the effect of potassium chloride, repeating the experiment of the previous afternoon with sodium chloride. The 10 c.c. of milk with 1 c.c. of distilled water became curdy at 69 and clotted at 70. The 10 c.c. containing 2 c.c. of distilled water became curdy at 68 and clotted at 70. The 10 c.c. with 1 c.c. of potassium chloride 10 $^{0}/_{0}$ solution became curdy at 82 and very curdy at 85. The 10 c.c. containing 2 c.c. of potassium chloride solution became curdy at 85 and very curdy at 87.

As is the case with clotting of blood, and the clotting of milk with rennet, potassium chloride is in this respect a little weaker than sodium chloride. Hence milk differs from caseine dissolved in lime water in respect to the effect of sodium chloride, for sodium chloride exerts no influence on the clotting of caseine by calcium chloride.

I next experimented to learn how sugar of milk influences the clotting of milk by calcium chloride.

I obtained some fresh milk faintly acid in reaction. To 10 c.c. of this I added three drops of calcium chloride solution, I then diluted 15 c.c. of milk with 45 c.c. of distilled water and to 10 c.c. of this I added three drops of calcium chloride solution and to another 10 c.c. in addition to three drops of calcium chloride solution, I added ·5 grammes of sugar of milk and placed the three test tubes in a water bath and heated it. The diluted solutions both became curdy at 70 whilst the undiluted milk curdled between 82 and 85° C.

From this experiment it would appear that sugar of milk has no influence in promoting or retarding the effect of calcium chloride on milk, in this respect milk differing from the caseine solution in lime water.

In the preceding experiments by diluting milk we of course lessened the percentage amount of sugar of milk, as it is quite possible that a small quantity of sugar of milk may possess the full effect in assisting calcium chloride to precipitate caseinogen or caseine, and that consequently increasing the quantity does not increase the effect. I therefore made the following experiments.

I took four specimens of 10 c.c. of caseine dissolved in lime water. To the first I added ·2 grammes of sugar of milk, to the second ·5 grammes, to the third 1 gramme. The fourth I used as a control; on placing the test tubes in the bath and heating it I found the third specimen began to grow milky at 34, the second at 45, and the third at 54, and the fourth at 68.

I then repeated this experiment but added to each tube two drops of calcium chloride solution. The third specimen containing most sugar of milk began to grow cloudy at 20, the second at 30, the first at 41, and the fourth without sugar at 50.

From this experiment it appears that increasing the quantity of sugar to about double the quantity contained in milk increases the effect of the sugar in assisting lime salts to precipitate curd from a solution of caseine in lime water. It would appear then that the dilution experiments are valid and so we may conclude that sugar of milk does not aid lime to precipitate caseinogen, whilst it acts powerfully on caseine. To avoid however any possible error I dialysed some milk for 47 hours. It still contained a little sugar as tested by the copper test. Some water got into the dialyser, for 100 c.c. of milk was increased to 300 c.c.

April 18. To four specimens of dialysed milk I added three drops of calcium chloride solution. To the second test tube I added ·5 grammes of sugar of milk. To the third ·5 c.c. and to the fourth 1 c.c. of sodium chloride 10% solution. The first with only calcium chloride curdled at 66. The second containing in addition half a gramme of sugar of milk curdled at 65. The third at 85, and the fourth at 94.

Sugar of milk then does not affect in any way, neither retarding nor accelerating, the curdling of milk by calcium chloride. Contrasting strongly in this respect with the influence of sodium chloride which greatly retards the action of calcium chloride. In other experiments I found a gramme of sugar of milk to 10 c.c. of dialysed milk did not affect clotting.

I find that sulphate of magnesia affects caseinogen and caseine differently.

April 19. Ten cubic centimetres of fresh faintly-acid milk with three drops of calcium chloride solution clotted at 74. A similar mixture of milk and calcium chloride with ·5 c.c. of 10 °/₀ solution of magnesium sulphate curdled at 74: with 1 c.c. of solution at 77, and with 2 c.c. at 85.

Sulphate of magnesium then in some degree antagonizes the action of calcium chloride on caseinogen, though as is well known in a saturated solution magnesium sulphate precipitates caseinogen.

We see then that caseine dissolved in lime water and caseinogen in milk, have some properties in common, but differ strikingly in others. Both are precipitated by calcium chloride, but caseinogen requires a higher temperature than caseine.

Sodium chloride, potassium chloride and magnesium sulphate antagonize the action of calcium chloride on caseinogen, a higher temperature being required with the presence of one of these salts to enable calcium chloride to clot caseinogen, while sulphate of magnesia greatly helps calcium chloride to clot caseine, and sodium chloride and potassium chloride are without effect. Further, sugar of milk greatly favours the clotting of caseine but does not influence the clotting of caseinogen.

On the behaviour of Caseinogen with Lime and other Salts.

I have followed Hammarsten's method in preparing caseinogen; and the addition of lime water and phosphoric acid and rennet he recommends, in order to clot the solution, but with very indifferent success.

Caseinogen prepared however in the way I am about to describe gives constant and uniform results with rennet and other salts.

Add acetic acid, 10 °/₀ solution to milk, wash the precipitate and filter and wash the curd deposited in the filter till it gives a neutral reaction and no precipitation on the addition of a solution of oxalate of ammonia. Rub the curd in a mortar with pure carbonate of lime, and then add distilled water. The caseinogen rapidly dissolves and the butter separates and floats on the top. After standing some hours syphon off the milky fluid.

This fluid gives an abundant deposit with acetic acid, is amphoteric in reaction and looks like skimmed milk. With reagents it behaves like milk.

Rennet clots this solution if a small quantity of lime chloride is added. I get this result only with Crosse and Blackwell's rennet, a strong saline neutral solution. I have not yet succeeded with rennet extracted by glycerine.

Like milk lime salts accelerate clotting, the larger the quantity of lime, the more rapid and complete the clotting, and like milk too, its clotting by rennet is opposed by sodium chloride, but the sodium chloride is antagonized by calcium chloride. With sufficient sodium chloride no clotting occurs, and if the sodium chloride is insufficiently antagonized by calcium chloride the clotting is delayed, and the clot is small and loose, and after contracting, the fluid above the clot gives an abundant deposit on the addition of acetic acid, showing that much of the caseinogen remains unprecipitated. Now this is not the case when lime chloride and rennet are alone added to the fluid, for then nearly all the proteid matter clots out of the fluid, so that on the addition of acetic acid little or no deposit occurs.

Ten c.c. of this solution with six drops of 10 $^0/_0$ solution of calcium chloride and ten drops of Crosse and Blackwell's rennet heated to 40° clots firmly in about five minutes, and the clot rapidly shrinks.

One cubic centimetre of 10 $^0/_0$ solution of sodium chloride, with 10 c.c. caseinogen solution and six drops of rennet, is antagonized by twelve drops of calcium chloride solution, a firm contracting clot ensuing, whilst 5 c.c. sodium chloride solution is antagonised by six drops of calcium chloride solution.

Potassium chloride acts like sodium chloride.

Sodium bicarbonate like sodium chloride antagonizes the action of both lime chloride and rennet.

To 10 c.c. of solution of caseinogen I added 6 drops, of 2 $^0/_0$ solution of sodium bicarbonate, and of rennet and of calcium chloride solutions and heated to 40°; no clotting occurred, but a control experiment without sodium bicarbonate clotted in a few minutes.

From these experiments it will be seen that perfect clotting can be produced without the addition of phosphoric acid. The commercial rennet contains a most minute trace of phosphoric acid, and not more probably than will be found in every solution of rennet. If phosphoric acid is essential perhaps this minute quantity sufficed.

If only a small quantity of lime chloride is added, as three drops of the solution to 10 c.c. of caseinogen solution, rennet only precipitates a part of the proteid, and on pouring off the supernatant fluid and adding acetic acid, an abundant deposit falls.

Crosse and Blackwell's rennet added to the caseinogen solution will not clot it unless a lime salt is added. The rennet contains a good deal of lime, but this is either insufficient, or in inappropriate form. But though the fluid does not clot, the caseinogen is converted into caseine which apparently remains in solution, and this is at once deposited by adding calcium chloride, each drop producing an abundant deposit which is insoluble in solution of sodium chloride.

Sodium and potassium salts not only delay clotting by rennet, but they lessen too the contraction of the clot with the solution of caseinogen, rennet aided by lime forms a clot which rapidly contracts till it does not occupy a tenth or twelfth part of the fluid, and the clot is firm and compact. If a sodium or potassium salt is added the clotting is delayed and the clot contracts slowly and slightly, as the following experiment shows.

To 10 c.c. of caseinogen solution I added six drops of rennet, six drops of calcium chloride solution. To another 10 c c. I added the same quantity of salt and rennet, in addition six drops of $2^{\circ}/_{0}$ of sodium bicarbonate and heated to 40. Next day the specimen without sodium bicarbonate contained a small firm clot whilst the specimen with sodium bicarbonate was set into a jelly, with very little contraction.

This experiment shows that the bulkiness of the clot in milk curdled by rennet depends on the potassium and sodium salts antagonizing in some measure the small quantity of calcium salt in milk.

Calcium chloride solution added in sufficient quantity to the caseinogen solution precipitates curds without the assistance of rennet. Thus 6 to 12 drops of calcium chloride solution to 10 c.c. causes an abundant curdy deposit at the temperature of the room. I thought at one time this might consist of caseine, the lime being able to convert caseinogen to caseine, without the help of rennet and especially when heated, but this deposit is not caseine, for it dissolves freely on adding a strong solution of sodium chloride, and this is not the case with caseine.

This solubility in solution of common salt explains the change that occurs in caseinogen solution on the addition of Crosse and Blackwell's rennet which contains a good deal of common salt, for on adding ·5 c.c. of this rennet solution to 10 c.c. of caseinogen solution, the fluid becomes clearer and less milky, which suggests that the solution of caseinogen is not quite perfect.

Lime chloride solution acts in the opposite way to sodium chloride and increases the milkiness, and if enough is added precipitates curd If insufficient to precipitate curd is added on heating, the fluid becomes very milky, but on cooling the milkiness becomes less again, suggesting that as is the case probably with caseine, the caseinogen combines with lime forming a compound behaving like many other lime salts in being more soluble at a low than a high temperature.

Sulphate of magnesia like common salt hinders or prevents the action of calcium chloride.

I took 10 c.c. of caseinogen solution and added six drops of calcium chloride solution, and curds deposited at the temperature of the room and the fluid became quite thick at 40° C. To a second 10 c.c. I added ·5 c.c. of 10°/₀ solution of magnesium sulphate, and to a third 10 c.c. 1 c.c. of magnesium sulphate solution, and to both these I added six drops of calcium chloride solution and heated the fluids to 70° C. without causing any curdiness.

In this respect this solution behaves like milk and contrasts with caseine solution in lime water.

Sugar of milk is without effect on the precipitation of caseinogen by calcium chloride. I made a large number of experiments similar to those with caseine and milk, always with the same result.

If we may assume that the caseinogen prepared by this method is of the same composition as the caseinogen of milk, then these experiments show that one of the differences between caseine dissolved in lime water and milk depends on the effect of the salt in milk in antagonising lime chloride. We have seen that lime chloride can precipitate caseinogen from milk, but a higher temperature is required to precipitate caseinogen than to precipitate caseine dissolved in lime water. Now with caseinogen without any salts we find that lime chloride precipitates it much more readily than caseinogen in milk, though somewhat less readily than caseine in lime water, therefore the greater difficulty lime chloride has in precipitating caseinogen from milk is due in part to the salts of milk.

In other respects, simple caseinogen solution corresponds with milk with both sodium and potassium salts, and also magnesium salts oppose the action of lime salts, and sugar of milk is without influence on the action of lime chloride. In this last investigation too we find that caseinogen deposited by lime chloride is largely soluble in solution of common salt, whilst caseine precipitated by lime chloride or by rennet acting on milk is insoluble.

Caseine differs from caseinogen in yet another particular. Caseinogen precipitated by acetic acid, and mixed in a mortar with carbonate of lime is freely soluble in distilled water. Caseine treated similarly with carbonate of lime is quite insoluble in distilled water.

In the curdling of milk then by rennet, we have first the change from caseinogen to caseine, next the clotting of caseine by its combination with a lime salt; the precipitation of this compound is largely assisted by the sugar of milk, but opposed by the sodium and potassium salts, these retarding the clotting (precipitation) and lessening the degree of contraction, and hence we get a large bulky clot instead of a small more compact one.

I have also experimented with caseinogen, prepared by saturating milk with common salt, washing the precipitate with a saturated solution of sodium chloride and then adding the curd to distilled water. The caseinogen easily dissolves.

This solution behaves just like milk. It clots with rennet. To 100 c.c. I added 5 c.c. of Crosse and Blackwell's rennet and heated some to 40° C. It quickly clotted, and next day the rest which had not been heated was clotted. The addition of solution of calcium chloride accelerates the clotting. Calcium chloride with the aid of heat curdles the solution but requires a higher temperature than milk, no doubt owing to the large quantity of common salt in the solution. Sugar of milk and sulphate of magnesium do not assist calcium chloride in clotting the solution.

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FURTHER OBSERVATIONS ON THE BEHAVIOUR OF CASEINOGEN. By SYDNEY RINGER, M.D., F.R.S.

In a previous paper[1] I describe a method of preparing caseinogen and some experiments made with it. In those experiments I used commercial rennet, made no doubt with tap water and commercial common salt, hence containing a considerable quantity of a lime salt. In the experiments (made in December and January) of this communication I employed rennet made by Mr Martindale, with distilled water and pure sodium chloride and free from any lime salt[2].

I prepared caseinogen by adding to a pint and a half of milk 90 c.c. of 10 °/₀ solution of acetic acid[3] and filtered through blotting-paper. Each day the curd was well rubbed up with distilled water and refiltered, and this treatment was continued till the fluid passing through the filter gave no precipitate with solution of oxalate of ammonia, and only reddened blue litmus as much as distilled water. I then rubbed the curd in a mortar with carbonate of lime and added a pint of distilled water.

Twenty-four hours after dissolving the caseinogen, two, four and six drops of calcium chloride[4] solution to 10 c.c. of caseinogen solution produced no curdy deposit at the temperature of the room. Heated to 30° C. the solution containing six drops of calcium chloride solution became faintly granular, but this quite redissolved on cooling the solution. On adding to each test tube six drops of rennet at 30° C. the solution in each test tube almost immediately clotted.

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[1] This *Journal*, Vol. XI. No. 6, p. 473.

[2] The following is his recipe. For every four ounces of the mucous membrane of the calf's stomach add four ounces of pure sodium chloride. Place the mixture in a jar and allow it to stand five days. Then pound well in a mortar and mix with thirty fluid ounces of distilled water with six ounces of rectified spirit. Percolate through muslin.

[3] I prepared another specimen, adding to the same quantity of milk 175 c.c. of the acetic acid solution. Both specimens of caseinogen gave the same results with lime and rennet.

[4] Throughout this paper I employed 10 °/₀ solution of calcium chloride.

This experiment clearly shews that it is not necessary to add phosphoric acid to induce clotting; and that a lime salt and rennet are sufficient.

Forty-eight hours after solution the caseinogen behaved the same with calcium chloride and rennet.

By keeping however, the solution changes, becoming more easily curded or clotted by calcium chloride. Thus on the sixth day, at the temperature of the room two drops slightly curded, and four drops abundantly curded 10 c.c. of the caseinogen solution, whilst with smaller quantities of calcium chloride and rennet, with the aid of heat, the solution still clots. On the seventh day two drops of calcium chloride solution precipitated abundance of curd without the aid of heat, and the solution was neutral in reaction. At the same time six drops of rennet added to 10 c.c. of the caseinogen solution at 30° C. curded the solution without the addition of calcium chloride[1].

On standing for many days the whole of the curd is deposited without the addition of any calcium salt, and the solution then strongly reddens blue litmus. The earlier changes before the precipitation of caseinogen are probably due to the formation of an acid, for we shall see that the addition of a minute quantity of acetic acid to milk, in a quantity quite insufficient to curd milk, strongly favours clotting by rennet and precipitation by calcium chloride.

If however the caseinogen solution be heated over a boiling water-bath and the flask stopped with cotton-wool, sterilized by being held a short time in a flame, the solution can be kept indefinitely without apparent change. I experimented with some solution kept more than six weeks and it behaved just like a freshly-prepared solution.

I next dissolved caseinogen prepared as before described in ·3 and ·4 °/₀ solutions of sodium bicarbonate. These solutions behave alike, differing only in the rapidity with which changes about to be described occur. Caseinogen freely dissolves in these solutions, and at first is milky, but in a few days, and much more quickly if the solution is boiled in a water-bath, the fluid becomes clear and of a light yellow colour. When first prepared this solution differs somewhat from the solution made with carbonate of lime, for it is less readily precipitated by calcium chloride and whilst clotting completely with the aid of calcium chloride and rennet, the clotting occurs more slowly.

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[1] This solution of caseinogen contains a small quantity of a lime salt, taken up when the caseinogen is rubbed with carbonate of lime. The quantity is insufficient to induce clotting with rennet in fresh solutions.

After a few days the solution clots less and less readily and abundantly, and still later large quantities as much as twelve drops of calcium chloride solution with six drops of rennet will not clot 10 c.c. of the solution at 45° C., neither will twelve drops of calcium chloride solution alone, although the temperature is raised to 90° C.

When freshly prepared, no clotting occurs with rennet unless a lime salt is added. In these experiments only calcium chloride was tested.

It was suggested to me that sodium bicarbonate converts caseinogen into alkali albumen and hence it could not clot with rennet. To test this suggestion, I precipitated the proteid with 10 °/₀ solution of acetic acid. Abundance of precipitate occurred. I filtered and washed the precipitate with distilled water till the acid was removed; then rubbed the deposit with carbonate of lime and added distilled water. After standing five hours I measured 10 c.c. with six test tubes and added calcium chloride solution in the following order, two, four, six, two, four, and six drops, and the last three test tubes each received six drops of rennet. At the temperature of the room no change took place. I then placed the tubes in a bath at 30° C. The tube containing six drops of calcium chloride became very granular, almost solid, but the deposit entirely dissolved on cooling the solution. No deposit occurred in the tubes containing four and two drops of calcium chloride solution. Each of the tubes containing calcium chloride solution and rennet clotted well and the clot rapidly shrank till it occupied only about a tenth of the fluid and was not dissolved on placing the tubes in iced cold water. It is obvious therefore that bicarbonate of soda does not change caseinogen into alkali albumen, but probably combines with caseinogen and makes the solution much more perfect and much more transparent, and at the same time antagonizes the action of rennet and of calcium chloride. In milk and in the solution of caseinogen by means of carbonate of lime the solution is much less perfect, and appears to be more of the nature of a perfect emulsion.

Sodium bicarbonate affects milk in the same way. Milk to which sodium bicarbonate, in the proportion of ·3 to ·5 °/₀, is added slowly changes and in the course of ten days or a fortnight will not clot, whilst it can still be precipitated by calcium chloride with the aid of heat. The proteid however can be precipitated by acetic acid, and if washed and mixed with carbonate of lime on adding four to six drops of calcium solution and a little rennet (six drops) and heating the fluid to 30° C. it quickly clots.

Milk treated with caustic soda ·2 % solution behaves in the same way.

Heat facilitates the change by sodium bicarbonate and caustic soda, but boiling milk for four hours without these salts induces no change, as it clots readily with calcium chloride and rennet.

The clotting of milk by rennet consists of two processes, and these can be dissociated, as the following experiment proves.

I prepared caseinogen by adding acetic acid to milk. The precipitated curd was filtered through blotting-paper and carefully washed with distilled water for several days. I then dissolved the caseinogen in ·3 % solution of bicarbonate of soda, and again precipitated the dissolved proteid with acetic acid and washed it well during several days, and again dissolved with ·3 % solution of sodium bicarbonate, and again precipitated the caseinogen with acetic acid and washed it well through a filter. The fluid passing through the filter gave no precipitate with oxalate of ammonia, and some of the caseinogen incinerated and tested with oxalate of ammonia gave no precipitate or turbidity. It was therefore free from a salt of lime. I then dissolved the caseinogen in ·3 % solution of bicarbonate of soda, and twenty-four hours afterwards poured 10 c.c. into a test tube, adding six drops of rennet and placed the tube for half-an-hour in a bath at 40° C. Next morning I again heated the solution to 40° and then placed the test tube in boiling water, the fluid neither curding nor clotting. This destroyed the rennet, as a control experiment proved. I then cooled the solution in water near the freezing point and poured it into a test tube containing four drops of calcium chloride solution without causing any clotting or curding. I then placed the test tube in water at 40° C. and in about a minute the solution clotted, on being cooled at the tap the clot dissolved, and when replaced in the warm water it again clotted, and the clotting by heat and solution by cold I repeated six times. I accompanied the preceding with a control experiment. I poured 10 c.c. of caseinogen solution which had not been treated with rennet into a test tube containing four drops of calcium chloride solution. No curding nor clotting ensued nor when the temperature of the fluid was slowly raised to 95° C.

In this experiment the rennet changed the caseinogen into caseine but produced no precipitation nor clotting. Then the rennet was destroyed by heat, and next after cooling the solution the addition of calcium chloride clotted the solution at a temperature about 30° C.

As the caseinogen was free from any lime salt it is obvious that the first process (gerinnung) takes place without the presence of a lime salt, rennet alone being sufficient, but no precipitation (abscheidung) occurs. This requires a lime salt, for on adding calcium chloride to the solution at 30° C. a well-marked clot quickly forms. Probably the caseine forms an insoluble lime compound, for the caseine behaves like many lime compounds in being precipitated by heat and dissolved by cold.

The same experiment succeeded even better with some of the caseinogen treated with rennet (100 c.c. to 4 c.c. rennet) and kept at a temperature of 40° C. for twenty-four hours. The caseinogen was more completely changed to caseine, and hence on adding 10 c.c. to four drops of calcium chloride solution and heating to 40° C. the deposit was more abundant. Much of the deposit dissolved at a low temperature.

The independence of the two changes is shewn in another way. I added 30 c.c. of Crosse and Blackwell's rennet and 20 c.c. of 10 °/₀ solution of calcium chloride to three pints of milk and placed it in a warm chamber at 40° C. After clotting I squeezed out all the whey and divided the curd into two parts; one I rubbed in a mortar with ·3 °/₀ solution of sodium bicarbonate, adding a pint and a quarter of the solution to the whey and heated the mixture in a flask over a boiling water-bath for half-an-hour and closed the flask with sterilized cotton wool. Next day, i.e. twenty-four hours after, most of the caseine was undissolved, and on filtering the supernatant fluid and adding a weak solution of acetic acid a very little curd precipitated. On adding 4 c.c. of calcium chloride solution to 10 c.c. and heating to 40° C. a very little curd was deposited.

I then doubled the quantity of bicarbonate of soda and next day rather more caseine was dissolved.

The other half of the curd I rubbed up with a weak solution of acetic acid, filtered and well washed till the fluid passing through gave no evidence of lime by oxalate of ammonia. This I then mixed in a mortar with ·3 °/₀ solution of bicarbonate of soda and poured into a flask and heated over a boiling water-bath. It quickly dissolved and five hours after all the curd was dissolved. On filtering the fluid and adding some weak acetic acid to 10 c.c. it became solid. I cooled 10 c.c. at the tap and poured it into a test tube containing four drops of calcium chloride solution. It became more milky but not curdy. On heating to 40° C. it clotted, becoming solid. On cooling at the tap the clot entirely dissolved and again the solution clotted on heating to 40° C. The solution without lime did not become in the least curdy on heating to 90° C.

In this experiment the acetic acid removed the lime salts and rendered the caseine soluble in bicarbonate of soda solution, which heat did not curd, but on adding calcium chloride solution and heating precipitation at once ensued and the fluid clotted as firm as milk.

I now record another curious effect of calcium chloride. To milk diluted with four parts of water I added rennet and placed the mixture for three hours in a chamber heated to 40° C. and then over a boiling water-bath. The rennet converted the caseinogen into caseine and this slowly settled. On shaking the mixture all the caseine became suspended, but in so fine a form that no particles could be seen. Yet when filtered the fluid passed through quite clear and all the caseine remained on the filter.

Two test tubes each received 10 c.c. of this suspended caseine. To one test tube three drops of calcium chloride solution were added and both were heated to 40° C. The fluid in the tube containing calcium chloride quickly became granular, then the granules aggregated with masses and all the caseine quickly settled, leaving the supernatant fluid quite clear. The other fluid without calcium chloride remained unchanged although the temperature was slowly raised to 90° C.

Six drops added to 10 c.c. of suspended caseine quickly caused aggregation and clotting at the temperature of the room, and one drop to 10 c.c. produced the same result in a solution heated to 90° C.

Here then calcium chloride caused the aggregation of the minute particles into masses.

The addition of a small quantity of acetic acid—too small to cause any precipitate—favours clotting. Two drops of 2 °/₀ solution of acetic acid to 10 c.c. of milk containing two drops of calcium chloride solution and six drops of rennet accelerated clotting. Some commercial rennets are acid in reaction and are reputed to be very rapid in their action.

We see a similar effect of a small quantity of acid in favouring coagulation of albumen by heat and in coagulation of myosin in rigor mortis. Probably the acid developed in a muscular contraction accelerates the partial clotting of myosinogen, though this effect is speedily obviated by the alkaline blood neutralizing the acid and so assisting to prevent complete and permanent coagulation, and so assists repair.

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THE INFLUENCE OF CALCIUM CHLORIDE ON EGG ALBUMEN AND SOME OF ITS DERIVATIVES.

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I.

In a previous communication[1] I record some experiments regarding the influence of calcium chloride on the coagulation of dilute serum, and in this paper I record the effect of calcium chloride on the coagulation of white of egg albumen.

In testing the effect of calcium chloride on egg albumen and some of its derivatives I made a series of experiments. Each series consisted of five experiments. In the five test tubes I dropped respectively 1, 2, 3, 4, and 6 drops of 10 °/₀ solution of calcium chloride and then poured in 10 c.c. of the albumen mixture.

The egg albumen was freely diluted, 25 c.c. being diluted to 200 c.c. with distilled water. I used a 10 °/₀ solution of calcium chloride[2] except when the contrary is stated.

I dropped the calcium chloride from a 5 c.c. pipette. Sixteen drops equalled 1 c.c.

The test tubes were placed in a cold water bath, and its temperature was raised by a Bunsen burner to boiling point.

Like Haycraft[3] I find that white of egg if freely diluted with distilled water does not coagulate even when heated to boiling point and only becomes a little milky.

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[1] This *Journal*, Vol. XII. No. I.

[2] Calcium chloride dissolved in water gives an alkaline reaction. In these experiments the alkalinity was neutralized by a weak solution of hydrochloric acid till the solution gave only the faintest trace of alkalinity.

[3] Haycraft and Duggan, *Brit. Med. Journal*, 1890. Vol. I. p. 167.

If however calcium chloride in sufficient quantity is added, it coagulates and the solution becomes at first particulate and then the particles cohere and form a firm clot. The temperature at which the solution becomes first particulate and then clots depends on the amount of calcium chloride and, as Haycraft has already pointed out, on the rapidity with which the temperature of the solution is raised. Calcium chloride requires some time to produce its effects on dilute egg albumen and large doses act more quickly than smaller doses, consequently if the temperature is quickly raised coagulation[1] occurs with the larger doses before the smaller have had time to cause coagulation.

While the coagulation temperature depends on the quantity of calcium chloride, the limit of its action is soon reached and larger quantities have no greater effect. With the quantities used in these experiments no coagulation occurred with white of egg solution without the aid of heat, although the mixture was allowed to stand in some cases twenty-four hours and even longer.

I record one of many experiments.

After preparing the test tubes as described, I placed them in a cold water-bath and raised the temperature. All the solutions became cloudy at 62° C. the cloudiness rapidly increasing till the solution became milky. The solutions containing 3, 4 and 6 drops became particulate at 74° C. That containing two drops at 77 and that containing one drop at 87° C. All clotted at from 84 to 85 except the solution with one drop which clotted at 96° C.

It appears then that increasing the quantity of calcium chloride solution to three drops lowers the coagulation temperature slightly but a further increase is without effect.

In the preceding experiment the temperature of the water bath was raised from 12° C. to 97° C. in about fifteen to twenty minutes and the comparatively rapid raising of temperature brings out the effect of calcium chloride, for with small doses as I have said the action of calcium salt is slow. I made some experiments prolonging greatly the time occupied in elevating the temperature.

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[1] By coagulation I mean the formation of particles in the solution, in fact the particulate stage. The effect of calcium chloride on alkali albumen was very protracted. The fluids when unheated not becoming particulate in some instances for a day or two days. It is obvious then that in many cases where the solutions were heated soon after being prepared, that the expression, coagulation temperature, has reference only to those conditions, namely the effect of heat, immediately or soon after the admixture of the calcium chloride and albumen solution.

In the following experiment just an hour elapsed between the rise of the temperature from 15° to 97° C. I made the usual series of experiments. All became cloudy at 60, beginning with those solutions containing most calcium salt and these remained the most milky. All the solutions except that containing only one drop of calcium chloride solution became particulate at 65, whilst the remaining solution became particulate at 67. All the solutions clotted at 80° C.

The clots in these experiments did not contract.

It appears then that with the large quantities employed if sufficient time were allowed that the coagulation temperature is much the same.

I next tested if smaller quantities of calcium chloride have a more marked effect on coagulation temperature. I used a 2 % solution of calcium chloride and repeated the experiments.

I give two examples. In the first the temperature was quickly raised from 10·5° C. to 95° C. occupying exactly eighteen minutes. All the solutions containing calcium chloride became cloudy at 58. The control without calcium chloride at 60° C. The solutions became particulate in the following order. Those containing 6 and 4 and 3 drops at 70° C. and that with 2 drops at 95° and the solution with one drop and the control remained merely milky at 96° C.

I next repeated this experiment raising the temperature from 12° C. to 97 occupying fifty-four minutes. The solutions containing calcium chloride became cloudy at 59° C. and the control at 60° C. The solutions became particulate in the following order: the solution with six drops at 67, with four drops at 70, with three drops at 81, with two drops at 94, whilst the solution with one drop and the control remained only milky.

These solutions contracted somewhat especially if boiled for some time. The contraction was more marked in the solutions containing two and three drops of the calcium chloride solution. As the clots contracted they left a clear fluid which contained very little proteid matter. These experiments then show that the temperature at which a solution of egg albumen becomes particulate depends on the amount of calcium salt in the solution, varying more than 30° C., whilst the temperature at which the solution becomes cloudy varies very little, not more than a degree and a half.

Before particles appear, the solution becomes somewhat glutinous; this is most marked with the smaller quantities of calcium chloride with two drops of the 2 % solution at boiling point. The particles were small and not very numerous and the fluid when poured out on blotting paper was quite glutinous like boiled starch. The whole of the proteid however was particulate, for if filtered, the fluid passed through the filter quite clear and contained only a trace of proteid whilst a thick gummy matter remained on the filter. The calcium chloride was in too small a quantity to cause aggregation of the particles and clotting as the following experiment shows. After filtering I placed the glutinous matter in a test tube and added about 10 c.c. of distilled water and then shook up the mixture. The solution became milky. I divided the mixture into two parts, and to one added two drops of 2 °/₀ solution of calcium chloride and placed both test tubes in a cold water bath and quickly raised the temperature. The solution to which I had added calcium chloride became coarsely particulate at 70° C., the small particles aggregating, and at about 90° C. the fluid clotted and then contracted, the contraction becoming considerable after boiling for some minutes and the fluid became quite clear and free from proteid. The solution to which no calcium chloride was added remained milky and no subsidence occurred; four hours later the fluid still remained milky.

It is obvious from this experiment that one effect of calcium chloride is the aggregation of fine particles into larger ones and then the formation of a clot and subsequently the contraction of the clot.

For with the first small quantity of calcium chloride even at a temperature of 96 to 100° C., the fluid only became finely particulate and glutinous; but after filtering and resuspending in distilled water a further addition of a small quantity of calcium chloride, with the aid of heat, formed a well marked clot which contracted greatly.

Barium chloride acts much the same as calcium chloride.

I made a similar series of experiments. The solutions containing 2, 3, 4 and 6 drops of 10 °/₀ solution became particulate at 71 and clotted at 80, whilst the solution containing one drop of barium chloride solution and the control solution only became rather milky at 96° C. So that this solution acted almost exactly as calcium chloride except that with the smallest quantity it did not coagulate the fluid but only made it milky.

Magnesium sulphate too acts on white of egg just like calcium chloride and barium chloride.

I again made a similar series of experiments. The solutions containing 3, 4 and 6 drops of 10 °/₀ solution became particulate at 80, the solution containing 2 drops at 83. The solutions containing 4 and 6 drops became solid at 80, the solution containing 3 drops at 88° C.

and the solution containing 2 drops at 95° C. The solution containing only one drop and the control only became milky at 96° C.

Haycraft and Duggan[1] find that the greater the dilution the higher the coagulation temperature, and they ascribe this effect to the dilution of the proteid. As however the addition of a calcium or barium or magnesium salt induces coagulation, it is evident that dilution of the proteid is not the sole factor. It would appear that dilution of the calcium salts is one cause. I have elsewhere shown that the influence of calcium and potassium salts on the contraction of the frog's heart depends on the percentage amount of these salts in the circulating fluid, that with diluted solutions their influence becomes less and these experiments I submit show that the same is the case with the coagulation of egg albumen by heat.

Haycraft and Duggan consider that cloudiness marks the beginning of the coagulation and that the minute particles, which are only visible by aid of the microscope, aggregate and become visible when the solution becomes particulate. They find that dilution only slightly raises the temperature at which the solution becomes cloudy, the difference between undiluted egg albumen and albumen diluted with water four times being only 3·75° C. whilst dilution greatly affects the particulate stage. The differences between undilute albumen and albumen diluted three times amounting to 16° C. whilst albumen diluted four times does not become particulate, but remains cloudy.

In my experiments with varying quantity of calcium salt, the temperature at which the solution became cloudy was very constant at 58° C. to 59° C. whilst the control solution to which no calcium was added became cloudy generally at 60° C. On the other hand the temperature at which the solutions became particulate varied widely, a difference as great as 30° C. or greater occurring between the solutions containing the larger, and those containing the smaller quantities of calcium salt.

I am sorry that my observations do not confirm Haycraft's statement that in the cloudy stage the fluid is particulate by the microscope. I have often examined the fluid with high power at the cloudy stage but without detecting any particles. It is true that later on at a higher temperature particles can be seen with the microscope before they can be detected by the naked eye. The changes appear to be these, first the fluid becomes particulate but the particles can only be seen through the microscope, next the fluid becomes viscid, and particles can be seen by aid of the microscope, next they become visible to the naked eye, and then aggregate into larger particles and at last the fluid clots into a jelly. The fluid may remain in any of these conditions even at boiling point, this depending on the amount of calcium salt in the solution.

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[1] Haycraft and Duggan, *Brit. Med. Journal*, 1890. Vol. I. p. 167.

II.

We have seen that dilute white of egg unboiled added to even large quantities of calcium chloride solution, namely six drops of the 10 °/₀ solution to 10 c.c., and allowed to stand several days undergoes no visible change, it neither becomes opalescent nor particulate. It is otherwise with dilute white of egg slowly heated and boiled for a few minutes. Ten cubic centimetres added respectively to 6 and 4 drops of calcium chloride solution and kept at the temperature of the room make the solution first viscid or glutinous and then it becomes particulate and at last forms a loose clot. With six drops this sometimes happens in ten minutes or with some solutions it is delayed for some hours whilst with four drops the solution may not become particulate for one or two days.

The white of egg albumen has been changed by boiling and is converted into alkali albumen, for as we have seen it is not coagulated by heat and it is precipitated by an acid and is dissolved by excess.

These solutions of egg albumen (alkali albumen) vary somewhat in respect to the behaviour with calcium chloride. With some solutions less than four drops will not cause coagulation at the temperature of the room, with other solutions it is different as the following experiment shows.

In the following series I employed 1, 2, 3, 4 and 6 drops of calcium chloride to 10 c.c. of the solution and obtained the following results.

In about ten minutes the solution containing six drops became particulate. In twenty to thirty minutes the solution with four drops became particulate. The other solutions after an hour remaining liquid and clear. Next morning, i.e. eighteen hours later, the tube containing three drops was particulate and in twenty-four hours the tubes containing 1 and 2 drops had undergone a similar change and the tube containing six drops was semi-solid.

The effect of calcium chloride then at the temperature of the air is with small doses very slow and prolonged and the clots that are formed are very loose and soft, and with some solutions the coagulation does not extend beyond the particulate stage.

If the solutions are heated immediately after mixing with the calcium chloride solution the coagulation temperature is found to be much lower than that of unboiled white of egg.

I diluted 35 c.c. of white of egg to 260 c.c. with distilled water and placing the flask in a cold water bath, slowly raised the temperature to boiling point and continued the boiling for four minutes. I quickly cooled the solution, which was quite milky but not particulate nor clotted. Into five test tubes I dropped respectively 1, 2, 3, 4 and 6 drops of calcium chloride solution and poured into each tube 10 c.c. of the white of egg mixture, and immediately placed the tubes in a cold water bath and rather quickly raised the temperature. The solution with six drops of calcium chloride became particulate at 33, with four drops at 39, with three drops at 46, with two drops at 59, and with one drop at 84. The solution with three drops clotted at 60, with two drops at 84 and with one drop at 90. The clots were firm especially with the larger quantities of calcium chloride. The clots did not contract.

I then performed the following experiment which shows that boiling for a longer time causes the solutions to clot quicker and more firmly.

I diluted 35 c.c. of white of egg to 250 c.c. with distilled water and slowly raised the temperature and boiled for fifteen minutes. The solution became very milky, and then made similar experiments as with the other solution. The solution containing six drops of calcium chloride without being heated clotted almost immediately, and in a few minutes later the solution with four drops and in about twenty minutes the solution with three drops. After an hour the other two solutions, containing respectively one or two drops, remained unchanged, but next morning, i.e. sixteen hours later, these also had clotted and all the clots were firm.

To a similar set of solutions I applied heat and the tube containing three drops became particulate at 40, that with two at 48° C. and that with one drop at 80 and all soon became solid.

In both series of experiments the clots did not contract.

I next tested the effect of heat on solutions with smaller quantities of calcium chloride using 2 % solution. The mixture containing six drops of calcium chloride solution became glutinous and particular at 88 and semi-solid at 92, the rest remained unchanged at 96 and were removed from the bath. The solution with four drops became viscid and glutinous about a minute after being removed from the bath. The solution with three drops became viscid eight minutes after removal from the bath, and remained viscid and transparent an hour later. The solution containing two drops became a little viscid fifty minutes after removal from the bath. The remaining solution with one drop was unchanged when I left the laboratory. Next morning the various solutions were in the same condition as on the previous afternoon.

If however the boiled albumen mixture is allowed to stand twenty-four hours it undergoes a slight change so that it will not coagulate at the temperature of the room, and the coagulation temperature becomes higher and after clotting, the clots contract slightly.

I made the usual series of experiments. The solutions became particulate in the following order, beginning with the solution containing six drops of calcium chloride solution, 28° C., 42° C., 47° C., 63° C. 90° C. and the clots contracted slightly, the contraction being most marked in those solutions containing most calcium chloride.

If the white of egg solution is boiled with a weak alkali, it coagulates and clots less rapidly, but the clots greatly contract.

I diluted 35 c.c. of white of egg to 250 c.c. with distilled water and added a gramme of sodium bicarbonate. The temperature was slowly raised and the solution boiled in a water bath for five minutes. It was filtered and quickly cooled and at once used in the following experiments. The solution was much less milky than those heated without sodium bicarbonate. In one series I slowly raised the temperature in a water bath. The solution with six drops became particulate at 42 and clotted at 80. The solution with four drops at 55 and 83° C., that with three drops at 70 and 86° C., whilst the solutions with one and two drops remained unchanged at 96° C. The clots greatly contracted, not occupying more than a tenth of the test tube.

A similar series were unheated and after an hour were unchanged. Four hours later the solution with six drops was slightly particulate and that with four to a still smaller degree. The other solutions were unchanged.

With this alkali albumen then, whilst the coagulation temperature with a given quantity of calcium chloride is higher than with the previous alkali albumen, and with small quantities no clotting at all occurs even at boiling point, yet the clots produced contract very greatly and all the proteid is precipitated, for if the fluid is filtered and tested it contains but a trace of proteid matter.

With a stronger alkali the alkali albumen will not clot even at boiling point with the quantities of calcium chloride used in these experiments.

To 200 c.c. of the 12·5 °/₀ solution of egg albumen I added 0·2 grammes of caustic soda and slowly raised the temperature in a water bath and boiled for ten minutes. The solution remained clear. Immediately it was cool I performed two sets of experiments, in one heating the solutions; in the other allowing them to remain unheated. Neither solution changed in any degree although in one series the temperature was raised to 96° C.

The alkali albumen however is coagulable by calcium chloride and heat, the coagulation being prevented by the antagonising action of the caustic soda preventing the action of calcium chloride, for on neutralizing the solution with weak hydrochloric acid, the solution containing six drops of calcium salt became cloudy at 30° C. and particulate at 50° C., the solution with four drops at 44° C. and 61° C., the solution with three drops at 56° C. and 78° C. whilst the solution with two drops became cloudy at 78 and remained milky and not particulate at 97; the solution with one drop was unchanged at 97.

The solution with 3, 4, and 6 drops clotted and the clots greatly contracted.

Even when treated with much larger quantities of caustic soda, alkali albumen acts in the same way, that is after neutralizing the caustic soda with hydrochloric acid the solution clots with calcium chloride and heat and the clots greatly contract.

The behaviour of alkali albumen with calcium chloride depends however on the time, and the temperature at which the albumen is treated with caustic soda.

To 85 c.c. of 30 °/₀ solution of white of egg I added 2 c.c. of 4 °/₀ solution of caustic soda and placed the flask in a water bath and quickly raised the temperature to boiling point and then removed the flask and quickly cooled the solution. I then neutralized the solution with 1 °/₀ solution of hydrochloric acid. This increased the albumen mixture to 200 c.c.

Of this I poured 10 c.c. into two test tubes containing respectively three and six drops of calcium chloride solution, both solutions became opalescent at the temperature of the room. At 54° C. the solution with six drops became particulate and at 75 the solution with three drops, both clotted well.

The alkali had converted the albumen to alkali albumen, for all the proteid was precipitated by an acid and dissolved in excess and on adding to the solution a little cupric sulphate solution and caustic soda solution it gave a violet colour.

I then boiled in a water bath a 30 °/₀ solution of albumen with 1°/₀ of caustic soda for a quarter of an hour and allowed it to cool gradually and stand for twenty-four hours. This solution gave the reactions of alkali albumen. I neutralized with weak hydrochloric acid and performed two similar experiments as recorded above but neither of the solutions became particulate though heated to 98° C. I diluted the solution to 25 % and repeated the experiments with the same result and I next made the solution decidedly acid but without causing any deposit and repeated the experiments, but without causing any precipitate.

Another specimen of diluted albumen of the same strength I treated with 1 % caustic soda for twenty-four hours at a temperature of 40° C. and then neutralized with weak hydrochloric acid. This solution gave no precipitate with similar quantities of calcium chloride though heated to 98° C.

It is obvious therefore that the prolonged action of caustic soda at a high temperature had produced some change in the alkali albumen, whereby it could not be precipitated by calcium chloride in quantities sufficient to cause firm clotting in albumen treated for a much shorter time with caustic soda.

The clots in all the previous experiments were to all appearance just like a milk clot or a fibrin clot. If they did not contract, they were in a jelly-like condition and when poured out of the test tubes retained their shape. When they contracted they looked more like a fibrin clot, the clot slowly leaving the lower part and sides of the test tube and is at last seen floating in a clear fluid.

In these experiments alkali albumen reacts very like caseine. For caseine[1] in a solution without a calcium salt or if this is present in too small a quantity remains in solution[2], but on adding calcium chloride the solution becomes granular and the granules aggregate into a clot that contracts greatly. This clotting takes place at the temperature of the room if enough calcium chloride is added; but with a smaller quantity the solution must be heated.

Caseine is an alkali[3] albumen; hence it would seem that the property of contracting may belong to the clots formed of alkali albumen, by the action of calcium salts.

I next tested the action of calcium chloride on Witte's peptone, which consists largely of albumoses with a little peptone.

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[1] By "caseine" I mean the curd produced by the action of rennet.

[2] This *Journal*, Vol. XII. No. II. 1891.

[3] If caseine is treated with acetic acid and then the curd is carefully washed with distilled water, the curd dissolves readily in 0·3 % solution of sodium bicarbonate. This solution does not curd on boiling, and is precipitated by adding a weak acid, the precipitate being soluble in excess of acid.

I made a similar series of experiments as with previous substances but found that no coagulation occurred even at boiling point.

I also experimented with Koch's peptones said to consist chiefly of albumose and with some gelatinose, etc.

After mixing a 10 % solution which gave an acid reaction, I made a similar series of experiments. The solution containing six drops of calcium chloride solution became particulate at 63 and the other solutions containing four, three, two drops and one drop in the following order at 67° C., 71° C., 76° C. and 78° C. The precipitate in each of the tubes remained particulate and was very small in quantity in the tubes containing one drop of calcium chloride solution, and increased with the increased quantity of calcium chloride, so that in the tube with six drops the deposit amounted to about a quarter of the bulk of the fluid. I filtered the solutions and saturated the filtered fluid with ammonium sulphate, a slight deposit occurred in the solution with the highest quantity of calcium chloride and this was larger in the other tubes in inverse order to the amount of calcium chloride being abundant in the solution with only one drop of calcium chloride solution.

Barium chloride and magnesium sulphate also precipitate Koch's peptones with the aid of heat, but the amount of precipitate is less with barium chloride than with calcium chloride, and is very slight with magnesium sulphate. Magnesium sulphate requires a higher temperature than calcium chloride to cause even a scanty precipitate. Six drops of a 10 % solution of either barium chloride, calcium chloride, or magnesium sulphate were unable to cause a precipitate in 10 c.c. of the Koch's peptone solution at the temperature of the room although allowed to stand for twenty-four hours.

It is obvious from these experiments that Koch's peptones contain a large quantity of proteid other than albumoses and peptones.

Soldner's[1] experiments lead him to agree with Hammersten that caseine (caseinogen) behaves like an acid and combines with lime. He believes that lime is made soluble in milk by combining with caseinogen. He describes three compounds, an acid, a neutral and an alkaline compound. The alkaline, neutral or only just perceptibly acid compounds do not coagulate on boiling. More acid combinations coagulate by heat, at a lower temperature the higher the acidity.

Milk made acid by acetic acid coagulates by heat, and calcium chloride favours the coagulation and clotting.

I added 20 c.c. of 1 % solution of acetic acid to 200 c.c. of milk. The milk appeared unaltered, it was not particulate even when examined with a microscope and passed through filtering paper. Moreover after standing twenty-four hours, no precipitate formed. With this solution I performed six experiments. Each tube contained 10 c.c. of acidulate milk. The first tube contained this alone, to the others I added solution of calcium chloride in the following order, one drop, two, three, four and six drops, and placed the tubes in a water bath slowly raising the temperature. The solutions became particulate at the following temperatures, beginning with the solution containing six drops of calcium chloride solution, 65° C., 68° C., 70° C., 75° C., 80° C. and 84° C. All clotted, those with most lime most firmly.

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[1] *Jaresbericht der Anatomie und Physiologie*, Vol. XVII. 1888, p. 314.

I have already shown that a calcium salt, after a solution has become particulate, causes the particles to aggregate into larger particles and then to cohere into a clot and that appears to be the action of the calcium chloride added to these solutions, for clotting occurred in the solutions to which no calcium salt was added. The following experiment shows that the calcium chloride only accelerated changes which could occur without its aid.

I repeated the six experiments recorded above. At 67 the solution with six drops of calcium chloride became particulate and I kept the temperature of the bath at this point. Ten minutes later the solution with four drops became particulate and after another ten minutes the tube with three drops, and twelve minutes later the solution with two drops was particulate. I then left the laboratory. Three hours later the solution with one drop and the solution of simple acidulated milk were also particulate. All these solutions to which calcium chloride was added clotted, whilst the acidulated milk remained particulate.

I now record some experiments which, as far as they go, support Soldner's conclusions and show the necessity of a calcium salt for the coagulation of milk by heat.

I precipitated caseinogen by adding to milk acetic acid. After filtering and well washing the curd I rubbed some in a mortar with carbonate of lime and then added distilled water; another portion I mixed with 0·5 °/₀ of sodium bicarbonate and allowed both mixtures to stand nineteen hours. I then syphoned off the solutions and filtered them. The caseinogen dissolved by aid of the calcium bicarbonate gave a slight acid but no alkaline reaction. I then added five drops of 1 °/₀ solution of acetic acid to 10 c.c. without making the solution particulate, although it gave a decided acid reaction. It passed readily through filtering paper leaving none on the filter. I placed the tube in a water bath and raised the temperature; at 80 the solution curdled. All the caseinogen was precipitated, for filtering and saturating the filter after with ammonium sulphate produced no deposit. A control experiment with unacidulated caseinogen solution neither became particulate nor clotted at boiling point.

I also added acetic acid to the solution of caseinogen in sodium bicarbonate solution. I made it strongly acid without causing any precipitate and yet on boiling could not make the solution particulate, but on adding a little calcium chloride solution the caseinogen solution became particulate and a copious deposit formed.

It appears therefore that whilst the acid compound of caseinogen with lime is precipitated by heat, the acid compound with soda is not precipitated even when boiled, but the addition of a small quantity of calcium chloride at once precipitates all the caseinogen.

As milk (caseinogen) has many of the properties of alkali albumen, I tested if alkali albumen behaves like milk when acidulated and boiled, i.e. whether it is coagulated when the acid has been added in too small quantity to precipitate the alkali albumen. I find that alkali albumen differs from caseinogen, for I could not coagulate it by heat till enough acid had been added to make the solution particulate so that the particles could be seen with a microscope, and the fluid filtered free from proteid matter, and this was the case also even when I added phosphate of soda before acidulating.

Influence of Calcium Chloride on Gelatin.

I obtained the gelatin of Mr Martindale. It was of French manufacture and of good quality.

I dissolved some gelatin in warm water and added various quantities of calcium chloride solution, then heated the tubes in a water bath to boiling point, without however inducing any cloudiness or coagulation.

I next boiled the solution of gelatin with some sodium bicarbonate and then tested the action of calcium chloride, but produced no change in the solution, even with the aid of heat. I then boiled the solution with caustic soda. This caused a flocculent deposit. I filtered this from the solution which remained when cold quite liquid and clear. I neutralized the solution with hydrochloric acid and then tested the action of calcium chloride and heat, but again induced no change. The solution contained a good deal of the gelatin, for on saturating with ammonium sulphate an abundant deposit occurred and this was freely soluble in hot water.

Gelatin therefore as regards the behaviour of calcium salts is quite different from egg albumen, serum, fibrin, muscle dissolved in weak caustic soda solution, and milk.

THE INFLUENCE OF CERTAIN SALTS UPON THE ACT OF CLOTTING.

By SYDNEY RINGER, M.D., F.R.S., AND HARRINGTON SAINSBURY, M.D., M.R.C.P., *Phys. Royal Free Hospital and Assistant Phys. Victoria Park Hospital.*

THE experiments about to be detailed were begun with the object of determining how the act of the coagulation of the blood might be influenced by the presence of certain salts. In the first place the enquiry was limited to the clotting of blood, but subsequently it was made to include the clotting of other fluids. Inasmuch, however, as the coagulation of blood is the most familiar instance of clotting, the experiments with this fluid have been the most numerous and they have formed the nucleus, as it were, of our investigations.

The problem of the coagulation of blood, as it now stands, recognizes three factors in the equation:

(1) a coagulable material,
(2) a ferment,
(3) certain salts.

Of these three factors, it is the first two which have attracted the greatest amount of attention. The last, it is true, has not escaped the observation of workers, either in the past[1] or more recently[2], but still, in comparison with the first two, it has been neglected, and hence we find even in the latest text-books of physiology, vague if not tentative statements with regard to the part played by salts in the act of clotting. We have sought to develop this third term.

The considerations which led up to this enquiry were the following:—There is between the act of muscular stiffening in rigor mortis and the act of congealing (of the blood) a close similarity. In either act there is the passage of a substance from the liquid into the solid state, this passage being accompanied as elsewhere by the evolution of heat; further there is the development of an acid, as shewn either by actual acidity or diminished alkalescence; thirdly, there is the appearance of carbonic acid[1].—So parallel indeed are the two processes in their details that to quote Prof. Foster we may "speak of rigor mortis as characterised by a coagulation of the muscle plasma, comparable to the coagulation of blood plasma but differing from it inasmuch as the product is not fibrin but myosin[2]."—But the setting of a muscle fibre in rigor mortis presents many resemblances to the change of state which a fibre undergoes when, during life, it contracts.—The analogy holds in respect of each of the above-mentioned points, e.g. the development of heat, of acidity, and the evolution in each case of carbonic acid. Moreover, though Foster insists that the analogy ends here, we would point out that the contracted muscle is denser than the uncontracted muscle, and that it has taken therefore one step towards the solid or less liquid state[3]. And again, that the act of contraction seems to prepare the way for rigor mortis—tetanizing a muscle facilitating its passage into the rigidity of death. On all these grounds therefore, even though we may admit the physical differences which actually do exist between the two states and may allow that there is no "satisfactory evidence of the formation during a contraction of any body like myosin[4], is there not some justification for Hermann's view that a contraction is a physiological and transitory rigor[5]?

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[1] Hewson—Davy. See Gamgee's *Physiological Chemistry of the Animal Body*, p. 54.

[2] A. Schmidt—Hammarsten, *opus cit.* Green, This *Journal*, Vol. VIII. No. 6. Freund—Latschenberger, *Cblatt. f. Phys.*, Oct. 26, 1889.

From the comparison between muscle in rigor mortis and muscle in contraction we pass directly to the comparison between the contraction of muscular fibre and the act of the clotting of blood.

Previous experiments with contracting muscle had shewn us that the presence of certain salts exerted a marked effect on the act of contraction. For instance, it was found that an artificial saline circulating fluid which should efficiently keep going the excised frog-heart must contain either calcium or strontium as one element. By an artificial saline circulating fluid, we mean a fluid composed of inorganic constituents alone. Further we had found that, in larger quantities, salts of potassium and salts of lime, introduced into an artificial blood mixture circulating through the excised frog-heart, were strongly antagonistic in their action upon the heart; thus, whilst the addition of potassium salts in increasing percentage would cause the contractions to grow weaker and weaker till finally there was complete abolition of spontaneous beats, and the ventricle moreover would give no response even to the strongest electrical stimulation, the addition of a lime salt would gradually remove this restraint and the ventricle would recover its excitability and respond to stimulation by vigorous contractions. Clearly the potassium salt in this experiment had so modified the muscular tissue that it had become incapable of passing through those molecular changes proper to the act of contraction. As clearly the lime salt had undone the work of the potassium salt—the interference was more or less complete. The molecular changes of contraction represent however, whatever view we take of their exact nature, a passage from higher to lower; they are catabolic. The muscular fibre before the act of contraction is a more vital, i.e. potential tissue than it is the moment after contraction. The restoration to the tissue of its former potency must indicate the exact reversal of this process; the molecules are again built up into a state of unstable equilibrium, the change is anabolic.

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[1] Strassburg (*Pfluger's Archiv*), Vol. VI. p. 65;
Tension of CO_2 in arterial blood = 21·28 mm. Hg.
Tension of CO_2 in venous blood = 41·04 mm. Hg.
Tension of CO_2 on coagulation = 61·79 mm. Hg.

[2] Foster. *Text-book of Physiology*, Ed. V., p. 99.

[3] Hermann we note urges this amongst other points in favour of the analogy between muscular contraction and rigor mortis. See Landois, Ed. VI., p. 579.

[4] Foster, *op. cit.*, p. 103.

[5] Hermann, *loc. cit.*

Regarding thus the process, the lime salt might be described as catabolic in its function, the potassium salt as anabolic. But however described, if the analogy between muscular contraction and clotting should hold, a similar relation of the salts of potassium and lime to the act of clotting was likely to obtain. We were thus led to anticipate the results actually gained.

Our method of experimenting has been as follows:—

To a series of flasks each containing a given quantity, 100 cub. cent. or 50 c.c. of the coagulable liquid, the salines have been added in ascending quantity. First, those salines which have a tendency to prevent the formation of clot. This series has been controlled by a flask containing the liquid of the experiment, without any saline addition. Next, and side by side with this series, another set of flasks, the exact counterpart of the series just described, with the addition however of a salt whose tendency is to favour clotting. In this second series, therefore, a double or antagonistic action is at work. In most cases this series also had its control, i.e. its flask containing the coagulable liquid plus the catabolic salt alone.

The salts we used were the chlorides of sodium and potassium and the chlorides of calcium, strontium and barium, and we worked with large quantities of these substances, adding for instance of the chlorides of sodium and potassium sufficient to make the coagulable liquid contain from 0·5 %—3 % or even 4 %.

Of the chlorides of lime and barium and strontium we used as a rule 2 c.c. of a 10 % solution for every 100 c.c. of the coagulable liquid.

Experiments with blood:

For the most part we have worked with the blood of the cat, but some few experiments were made with sheep's blood and bullock's blood. The results obtained have been so uniform that it will be unnecessary to describe in full more than one or two experiments out of each set.

Experiment VII. (Blood series)

The experiment was started at 10 a.m. on Dec. 7, 1889:—

A cat, completely narcotized with chloroform, was bled from the carotid and the blood received directly into a solution of sodium chloride, 0·6 % in distilled water. One volume of blood was added to 19 volumes of saline.

Into each of four series of flasks 100 c.c. of this blood mixture were poured, plus additional quantities of salts, viz.,

of sodium chloride sufficient to make

0·5 %, 1 %, 2 % and 3 % solutions. Series *A*.

of potassium chloride similar quantities. Series *B*.

The third row contained the same quantities of NaCl as series *A*, but each flask received in addition 2 c.c. of a calcium chloride solution of 10 % strength. Series *A'*.

The fourth row was a repetition of series *B* + 2 c.c. of the $CaCl_2$ solution in each flask. Series *B'*.

This experiment was controlled by a flask containing 100 c.c. of the simple blood saline.

The above percentage figures refer simply to the quantities of salt *added*, they do not take into account the 0·6 % NaCl present in all the flasks.

At the end of four hours. The flasks of series *A'* were all clotted and in the jelly-stage, with the exception of the 3 % flask.

Of series *B'* all the flasks were clotted; in the 0·5 % flask the clot was considerably contracted, in the 1 %, 2 %, 3 % flasks it was in the jelly-stage.

In the control and in *A* and *B* there was no clotting.

At the end of 24 *hrs.* There was still no clotting in the control nor in *A* or *B*; all these flasks had given an abundant corpuscular sediment.

In *A'* all the flasks were now clotted; the 0·5 % flask shewed considerable contraction of the clot, to not quite ¼ of the bulk of the fluid; the other flask little, if any, contraction of the clot.

In *B′* the clots in the 0·5 $^0/_0$ and 1 $^0/_0$ flasks had shrunk to small button-like masses of about equal size, whilst the 2 $^0/_0$ and 3 $^0/_0$ flasks shewed bulky clots about $\frac{11}{12}$ of the fluid volume.

After 48 *hrs.* There was still no clotting in the control and none in series *A*. In series *B* there was a doubtful flocculent separation in the fluid of the 0·5 $^0/_0$ flask; no change in the other flasks of this series.

In *A′* further contraction of the clot in 0·5 $^0/_0$ flask, rest very voluminous.

In *B′* slight further contraction of the clots, but these still voluminous in 2 $^0/_0$ and 3 $^0/_0$.

After 72 *hrs.* Still no clotting in control or in *A*. In *B*, 0·5 $^0/_0$ a slight clot.

After 96 *hrs.* A further doubtful clotting in the 1 $^0/_0$ flask of *B*. No clotting in the 2 $^0/_0$ or 3 $^0/_0$; none in control or in *A*.

After 120 *hrs.* A slight clot was seen in the control but none in *A*. No change in *B*.

Meanwhile the clots in *A′* and *B′* had undergone some further contraction but in the 2 $^0/_0$ and 3 $^0/_0$ they were still very bulky.

At the end of the 6th or 7th day (? which) the fluids were filtered and there was found: in the control a small quantity of loose clot in *A*, a small quantity of clot in 0·5 $^0/_0$ and 1 $^0/_0$; in the 2 $^0/_0$ it was doubtful; in the 3 $^0/_0$ there was none.

In *B* there was clotting in all the flasks, but it was minimal in the 3 $^0/_0$ and very slight in the 2 $^0/_0$; in the 1 $^0/_0$ and 0·5 $^0/_0$ the clotting was only slight.

This experiment, which as such or with slight modifications has been several times repeated and always with the same results, teaches:

(1) the delaying influence of the salts NaCl and KCl on the clotting process.

(2) that the sodium salt is rather more powerful than the potassium salt in this respect.

(3) the influence of lime in favouring clotting as shewn by its complete antagonism both of the sodium and potassium effect; and it must be observed not only that lime overcomes the influence of Na and K but that it also greatly accelerates the act of clotting. In the lime series again the greater restraining influence of Na than of K is shewn by the lime effect being most pronounced and appearing earliest in the series *B′*.

There are some other points which call for notice in this experiment, viz., the preservation of the red corpuscles—all the flasks clearing nearly completely by corpuscular subsidence, and also the great variation in the degree of contraction of the clot. To these points we shall refer subsequently.

On Dec. 10, 1889. Another experiment (VIII. in our series) was performed, which in all points was the exact counterpart of the one just described, excepting that we worked with a potassium chloride solution of 0·5 °/₀ strength instead of a 0·6 °/₀ NaCl solution. The cat's blood was diluted as in the preceding experiment twenty times.

5 hrs. after the commencement of the experiment all the lime flasks were in the jelly-stage, whilst of the non-lime flasks only the 0·5 °/₀ *B* was clotted. The control was thick but not distinctly clotted.

7 hrs. from the beginning, the control shewed a voluminous clot, but there was no other clotting in these non-lime flasks excepting in the 0·5 °/₀ *B*.

It was not till 72 hrs. had passed that some clotting appeared in the 1 °/₀ *B*, and that then some clotting also appeared in 0·5 °/₀ *A*.

At the completion of the experiment, after 120 hrs. (five days) the higher percentages 2 °/₀ and 3 °/₀ of *A* and *B* shewed practically no clotting and the lower percentages comparatively little. The clotting in *A* was decidedly less than in *B*.

In the firmness of the clot there was a striking difference between the lime and the non-lime series, the former being much firmer.

In this experiment the restraining action of KCl and NaCl over clotting is evident, especially in the higher percentages; and the restraint of potassium is seen to be less than that of sodium. This is well seen on comparing the controls of this and of the previous experiment. The influence of lime in facilitating clotting is strongly marked.

The amount of contraction of the clot was much less in the lime series of this last experiment than in the former. This we shall refer to later.

In the first experiment of the blood series, begun on Nov. 21, 1889, we worked with cat's blood diluted twenty times with 0·6 °/₀ NaCl, using this in two series of flasks.

The 1st series contained potassium chloride in ascending percentage from 0·5 °/₀—4 °/₀.

The 2nd series contained KCl in the same strengths plus a fixed quantity of $CaCl_2$, viz. 2 c.c. of a 10 °/₀ solution to 100 c.c. of the blood mixture.

The control to the 1st series contained the blood mixture only.

The control to the 2nd series contained the blood mixture + the lime.

Within 8 hrs. there was clotting in the control + lime, and in the 0·5 °/₀ and 1 °/₀ and ? in the 2 °/₀ flasks of the lime series.

Within 24 hrs. all the lime flasks were clotted, whilst only the control of the 1st series was clotted.

Only after 48 hrs. were filmy clots seen in the flasks of the non-lime series.

After 72 hrs. the fluids of series 1 were filtered, and upon now adding 4 c.c. of a 10 °/₀ solution of $CaCl_2$ to these flasks, fresh clotting took place in all the flasks except the 4 °/₀ and within 24 hrs.

On refiltering and adding 2 c.c. more of lime chloride a slight separation of clot was again observed, but only in the 4 °/₀ and 3 °/₀ flasks.

This experiment shews in yet another way the influence of lime in favouring clotting, and the restraining influence of KCl.

Having established the influence of lime in accelerating clotting, we next proceeded to test the influence of the allied elements strontium and barium. A 10 °/₀ solution of the chloride of each was employed. The experiments made were exactly similar to those just described, with the exception that the calcium chloride was replaced by strontium chloride and barium chloride. It will be sufficient if we give the results simply:—

Strontium chloride acts like calcium chloride but it is rather less powerful. The clots produced are much less contracted and definite[1].

Barium chloride acts like calcium chloride but it is less powerful. The clots produced appear later and contract less than for calcium.

In the strontium chloride and barium chloride experiments, the restraining influence of NaCl and KCl shewed itself as usual.

Experiments with pathological fluids.

The influence of sodium and potassium chlorides and of calcium chloride on the clotting of the fluid of ascites and of pleurisy has been tested in the manner already detailed. Six experiments have been made—five with ascitic fluids—one with pleuritic effusion. In three of the ascites cases there was associated disease of the kidneys—the other two peritoneal fluids were from the same patient (two different tappings) who was suffering from cirrhosis of the liver—the fluid in this case was slightly bile-stained. There was a great variety in the characters of the fluids, some being highly albuminous and becoming almost solid on boiling after acetification, whilst one was a thin opalescent fluid yielding but very little albumin to the same test. The spontaneous clotting of these fluids severally differed much. In three cases there was a marked tendency either to a slowly progressing clotting or to the repeated separation of clot—for repeated filtrations would be followed by repeated clotting. In some cases the clotting was bulky, in others very slight.

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[1] The cat's blood was received directly into a solution of NaCl 0·5 °/₀ as against 0·6 °/₀; it is probable that this difference in strength will in part account for the less definite character of the clots and the smaller degree of contraction. If this be so it will make Sr approach more nearly to Ca.

It was a curious fact noted more than once that fluids which were distinctly putrid would still be capable of clotting.

In spite of the very variable characters of these fluids the influence of lime in accelerating and of sodium and potassium in restraining clotting was distinctly manifest.

The restraint of potassium was less than that of sodium, and it was therefore more easily antagonized by the lime.

Experiments with milk.

We have repeated with milk the previous investigations. In the first instance we diluted the milk 10 times, but though in this dilution we obtained quite definite results, yet we found by a control experiment that far more striking results were to be obtained with undiluted milk, accordingly we used this thenceforward. In these experiments it was necessary to introduce another factor from outside for milk not being a spontaneously coagulating fluid, except by a process of acid fermentation which we were not anxious to induce, it was necessary to add the required ferment. We chose, for this purpose, an ordinary commercial essence of rennet, and this proved perfectly satisfactory.

We will give as a specimen the details of one experiment.

Jan. 27, 1890.

Fifteen flasks, each containing 50 c.c. of undiluted milk were arranged as follows:

Series *A*. Three flasks, each containing sodium chloride in the following percentages, 0·5 °/₀, 1 °/₀, 2 °/₀.

Series *B*. Three flasks, each containing potassium chloride in the following percentages, 0·5 °/₀, 1 °/₀, 2 °/₀.

Series *A′* the same as *A*, but in addition each flask received 1 c.c. of a 10 °/₀ solution of calcium chloride.

Series *B′* the same as *B*, plus 1 c.c. of a 10 °/₀ of $CaCl_2$.

The double saline series was controlled by a pure milk flask.

The double lime series was controlled by a milk + lime flask.

These 14 flasks each received 1 c.c. of the essence of rennet to render them spontaneously coagulable.

Finally to control the whole series of 14 flasks, the 15th contained pure milk only (i.e. milk without rennet).

The experiment was started at 10·30 a.m. The room was warm.

These were the results:

2 hrs. 50 sec. after. All the lime flasks with the doubtful exception of the 2 $^0/_0$ A' and B' were clotted; the jelly was firmest in the control + lime, next in the 0·5 $^0/_0$ A' and B', next in the 1 $^0/_0$ A' and B'; in the 2 $^0/_0$ A' and B' the milk if not already in the jelly-stage was certainly thicker than in the non-lime flasks.

No clotting in any of the non-lime flasks.

At 4 hrs. from the commencement of the experiment, all the lime flasks were clotted, none of the non-lime flasks were clotted, but the control of this series shewed thickening of the fluid.

At 7 hrs. the non-lime control was in a loose jelly.

A, 0·5 $^0/_0$ a loose jelly; 1 $^0/_0$ a loose jelly; 2 $^0/_0$ not clotted.

B, 0·5 $^0/_0$ a firmer jelly; 1 $^0/_0$ less firm; 2 $^0/_0$ not clotted.

The clotting in *A* and *B* was less firm in the 1 $^0/_0$ than in the 0·5 $^0/_0$.

Lime series as before; clotting in all much firmer than in the non-lime series. The clotting in the control here was so firm that the flask could be inverted without any escape. The 0·5 $^0/_0$ of A' and B' were also very firm, though not quite so firm as this. The flasks of B' shewed slight separation of whey.

At 23 hrs. the whole series of 14 flasks had clotted. The pure milk control was unclotted. The milk in the 2 $^0/_0$ *A* and *B* was in a semi-solid state.

At this stage most of the lime flasks shewed a separation of whey (contraction of the clot), the KCl flasks more than the NaCl.

The non-lime flasks shewed no separation of whey, i.e. no contraction of the clots.

The milk at this stage was still alkaline in reaction.

At 48 hrs. all the flasks smelt sour—the pure milk control was still unclotted.

Contraction of all the clots with further separation of whey—this more marked in the lime series. On turning out the clots there was a striking contrast between the firmness of the 2 $^0/_0$ A' and B' and the softness of the 2 $^0/_0$ *A* and *B*.

On Jan. 28th this experiment was repeated with the addition of a 3 % flask of NaCl or KCl to each series, *A*, *B*, *A′*, *B′*; in all there were 19 flasks.

The results obtained were precisely similar. The 3 % NaCl (*A*) was not clotted at the end of 24 hrs. though the 3 % KCl flask was.

At the end of 48 hrs. the 3 % NaCl was found clotted.

In all, four experiments with lime were made—each experiment gave the same result. The conclusions to be drawn were:—

Lime assists powerfully the clotting of milk by rennet.

The chlorides of sodium and potassium retard this clotting, and NaCl more effectually than KCl.

The delaying action of these salines is obviated by the addition of chloride of calcium to the liquid.

With strontium chloride and with barium chloride similar experiments were made. These substances were found to act like calcium chloride. They were however distinctly less powerful, clotting being considerably delayed. The same quantities of these chlorides were taken as of the calcium salt, the experiments being in every respect similar to the one described above.

We may at this point contrast the two fluids blood and milk in respect of their physical state, because they are so constituted as, in comparative experiments, to enable us to gauge the rate at which clotting has taken place. Blood holds suspended particles, the blood cells, which being heavier than the plasma slowly subside. Milk holds suspended particles, the oil globules, which being lighter than the suspending fluid gradually rise. If whilst one or other fluid is thus clearing itself the act of clotting takes place we have in the thickness of the layer of corpuscular subsidence or of supernatant cream a means of estimating whether the act of coagulation took place earlier or later in one of two or more flasks. This is of course not a practical test except in cases of considerable difference, but it was exceedingly well illustrated in our experiments, for in the lime flasks, and especially in the controls + lime and in the smaller percentage flasks, clotting would sometimes occur so early that there would be either no subsidence at all and no cream or but the scantiest layer of each, whilst all the non-lime flasks would shew a thick layer of corpuscles or cream.

It is of some importance therefore, in blood experiments, that the red corpuscles shall be preserved. This we have found to be a matter of considerable delicacy, very slight differences in the artificial plasma being sufficient to preserve or break up the red cells, the latter process taking place very rapidly. For example, cat's blood received into a sodium chloride solution 0·6 % is but little acted upon, as to its red cells, the fluid becoming almost colourless by their subsidence. On the other hand the same blood received into a 0·5 % of NaCl suffers rapid disintegration of its red cells with diffusion of their contents[1].

Other changes appear to be effected at the same time, for a fluid in which the corpuscles are thus broken up appears to clot more readily, i.e. sooner, but the clot does not contract like the clot formed in the 0·6 % NaCl blood: the fibrin or the fibrin yielding substance must have undergone some change[2]. We have several experiments on this point.

These are side issues—to return to the main question: the positive influence of lime in favouring clotting: the adverse influence of the salts of potassium and sodium on this act: the mutual antagonism of lime on the one hand and of sodium and potassium on the other.

First the influence of lime:

Hammarsten shewed that the addition of calcium chloride to some specimens of hydrocele fluid which would not clot on the addition of the fibrin ferment would cause the separation of more or less fibrin;[3] in fact would act like paraglobulin. Of the action of calcium chloride on a blood mixture capable of spontaneous clotting we have found no statement referred to him.

Green[4] more recently has taken up this latter point, at least so far as the element calcium is concerned. According to his investigations calcium is essential to the act of clotting in blood, but we are led to infer from his statements that the lime must be in the form of the sulphate of calcium[5]. On p. 365 he states definitely that calcium chloride is inoperative. Green certainly establishes the influence of calcium sulphate on coagulation. He worked with very small quantities.

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[1] We were working with blood diluted 20 times with such salines.

[2] Blood received into an 0·5 % KCl (one part of the former to nineteen of the latter) suffers rapid disintegration of its red cells—the fluid clots more rapidly but the clot contracts comparatively little. If a series of flasks be started with this saline, then one of these flasks will contain the following constituents: 0·5 % KCl + 0·5 % NaCl + $CaCl_2$, and this flask will have its counterpart in the series in which the 0·6 % NaCl was used, for one of these flasks will contain 0·5 % KCl + 0·6 % NaCl + $CaCl_2$, therefore practically identical quantities of salines—yet this latter will yield a good contracting clot, whilst the former whose corpuscles have been first broken up by the 0·5 % KCl yields a bulky, very indifferent clot.

[3] See Gamgee. *Phys. Chemistry*, p. 52.

[4] This *Journal*. Dec. 1887. Vol. VIII. No. 6.

[5] See in particular, p. 370.

Latschenberger[1] quotes Freund[2] to the effect that the separation of insoluble phosphate of lime, a constant element in the ash of fibrine, is the sole cause of the clotting of serous fluids. L. on the other hand finds that calcium chloride is as effective as a mixture of calcium chloride and rhombic phosphate of soda. He suggests that it is probable that without calcium salts clotting will not take place.

Our own experiments with calcium chloride leave no doubt as to the efficacy of this salt, and make it clear, we think, that we have here to deal with a generic action which will include, probably, all salts of lime in greater or less degree. The solubilities of the several salts of lime will probably control this to a great extent.

Next, have the allied elements strontium and barium, any resemblance to calcium in respect of an influence on clotting? Green answers this in the negative so far at least as the sulphates of strontium and barium are concerned. But whilst it is quite possible that this is so—we would point out that a negative statement in respect of such insoluble salts as strontium and barium sulphate would prove little[3]. Our own experiments prove that the chlorides of these three elements have a similar action. Hammarsten had already established this similarity of action for the clotting of milk[4], he moreover adds the allied element magnesium to the list of similars. We find that the similarity of action of barium, strontium and calcium holds for blood as well as for milk.

We should *a priori* be prepared to find that this action of barium and strontium is again a generic action. We have however no experiments on this point.

The restraining influence of the salts of sodium and potassium on clotting must now be considered. Schmidt drew attention to the importance of the presence of salts for the act of coagulation if only for the purpose of holding in solution the fibrin factors[5]. He appears to have found that the addition of NaCl up to 1 °/$_0$ to a weak sodium hydrate solution of the fibrin factors facilitated clotting. In our experiments we always found slight delay in clotting with this strength of sodium chloride which would correspond to our weakest sodium chloride flask, for the labelling of this flask 0·5 °/₀ simply indicated the additional amount of NaCl; there was already the 0·6 °/₀ NaCl present in the saline with which we diluted the cat's blood; the actual percentage of the flask would therefore be 0·6 °/₀ + 0·5 °/₀. It was however in the higher percentages that the restraint was most marked.

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[1] "Physiologie des Blutes." *Cbl. f. Physiologie*, Oct. 26, 1889.

[2] Wiener, *Medecin. Jahrb.* p. 259, 1888.

[3] $CaSO_4$ is soluble in cold water in the proportion of 1 : 400
$SrSO_4$,, ,, ,, ,, 3 : 10,000
$BaSO_4$ is insoluble. *Chemiker Kalendar.*

[4] Hermann. Vol. v. a. p. 54.

[5] Gamgee. *Op. cit.* pp. 53, 54.

It is of interest to note that the restraining action exerted by sodium salts is greater than for potassium salts: since sodium salts are *par excellence* the salines of the fluids of the body. It is also important to note that whereas the salts of these two bases stand near together by their action upon the clotting process they are very far apart in their action upon muscular contraction. This makes a flaw in the analogy between clotting and contraction.

The adverse influence of sodium and potassium chloride is opposed by calcium chloride. The establishment of this antagonism is the chief purpose of this paper. Here is an act, coagulation, which demands as essential factors the presence of a certain amount of a sodium salt, the presence of a certain amount of a lime salt; increase the amount of the sodium salt beyond a certain limit and the act of clotting is impeded, increase the amount of the lime salt and the restraint is partially or completely overcome[1]. Of what nature is the restraint, of what nature the favouring action? Sodium salts in general are both soluble and solvent, calcium salts in general are relatively insoluble and but little solvent, and it is tempting to seek a simple chemical interpretation of the problem and consider that we have in the one instance a relatively soluble compound of sodium with fibrinogen or with fibrin, in the other, a comparatively insoluble calcium compound with the proteid. There are however objections to this simple view. Green discusses the point in reference to the action of $CaSo_4$.

Then, inasmuch as the act of clotting must be regarded as one of descent from higher to lower, from less stable to more stable—an instance therefore of catabolism—the persistence in the fluid state may be regarded as one of upholding, a maintenance in a condition of higher potential, analogous to a building up: an instance therefore of anabolism. We may accordingly take the view that lime salts favour the process of degradation, whilst the sodium and potassium salts favour building up.

The antagonistic action of potassium salts and of lime salts on the contraction of muscular fibre, on the theory that contraction is analogous to clotting, would fit in with this view. But an objection here is that the action of sodium salts on the contraction of muscular fibre is far weaker than that of potassium salts, reversing therefore the relative action of these salts on clotting.

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[1] We did not push the quantity of lime to see if it had a far limit to its favouring action in excess of which, clotting became restrained or suspended.

It is possible that the view put forward by Gamgee, in explanation of the antagonism of certain salts in respect of clotting, will hold in some cases. He suggests for instance that when in certain cases the addition of $CaCl_2$ to hydrocele fluid + ferment sets up clotting the cause may be the decomposition of the alkaline carbonate present in this fashion:—

$$Na_2CO_3 + CaCl_2 = 2NaCl + CaCO_3.$$

His suggestion therefore amounts to chemical incompatibility as the cause of the antagonism. This may hold in cases where there is the separation of an insoluble substance, e.g. $CaCO_3$, but where after the addition of the antagonizing salt there is no such precipitation the explanation amounts to no more than the bare statement that in some way or other the salts do interfere with each other.

We must leave this matter here; there remains however to be said that when we speak of clotting we speak of a process liable to great variation and in different directions, e.g. the same amount of fibrin may be separated at very different intervals of time—rate of clotting; the same bulk of clot may include very variable amounts of fibrin: quantity of clotting, and conversely the same quantity of fibrin may occupy very different amounts of space, according to the degree of contraction of the clot.

We have dealt chiefly with rate of clotting and degree of contraction of the clot, and have not hazarded any judgment as to the amount of fibrin separated, excepting where equally bulky clots are unequally firm. The firmer clot in such case we have concluded to contain at least as much fibrin as the less firm. This question has not really been touched upon in the paper; we mention it only in order that it may not be thought that this aspect of the question has been overlooked by us.

Rate of clotting and degree of contraction we have found to vary remarkably in our experiments. The firmest clots and the most contraction clots we have obtained with the following saline combination.

Blood saline (1 vol. of blood—19 vols. of 0·6 % NaCl).
0·5 % KCl and 1 % KCl.
2 c.c. of a 10 % solution of calcium chloride for every 100 c.c. of blood saline.

In some cases the 1 % KCl flask gave the most rapidly contracting clot, in others the 0·5 %, but either rapidly followed the other's suit, and the ultimate degree of contraction was about the same for both.

Other things being equal we conclude that the act of clotting is most efficiently performed the firmer and the more contracted the clot produced, and accordingly we conclude further that a number of constituents share and are necessary to the act of clotting, and that for the greatest efficiency of the act definite quantitative relation must exist between these constituents. Till this quantitative relation is reached it is possible that the saline constituents cooperate with each other, and that antagonism comes in only when this limit has been overstepped.

To sum up our results and conclusions, they are as follows:—

(1) Confirmation of Green's statement that calcium is an essential to the act of clotting.

(2) Amplification of his statement by the determination that calcium chloride is a very efficient salt in favouring clotting, and conclusion therefore from Green's, Latschenberger's, Freund's and our own experiments that the effect of calcium is a generic effect belonging probably to all the salts of calcium.

(3) Determination that strontium and barium act like calcium but are less powerful.

Suggestion that this action will probably be found to belong to the salts of strontium and barium generally: the action of course being controlled for these bases as for calcium by the solubilities of the salts.

(4) Determination of the restraining action of potassium and sodium salts: the potassium effect being less than the sodium effect.

(5) Antagonism of the salts of lime, strontium and barium on the one hand, and of potassium and sodium on the other.

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THE ACTION OF SALTS UPON HEAT COAGULATION.

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In a previous paper on the influence of certain salts upon the act of clotting[1] a fluid capable of spontaneous coagulation was selected, and to this fluid salts in varying proportions were added. In this way it was sought to demonstrate the important part played by salts as agents favouring or retarding coagulation, and also that more or less antagonism or interference resulted from the simultaneous admixture of certain of these salts. The cooperative influence of salts was suggested by some of the experimental results obtained, according to which a maximum effect (as measured by the rapidity of the act of clotting and the degree of shrinkage of the clot) was witnessed when sodium, potassium and calcium chloride were added in certain proportions. This question was not, however, followed up.

The experiments about to be detailed form a natural sequence to those of the above investigation. The coagulation of certain proteids by heat was the process now examined into, the question being :—how the presence of salts might affect this process. Into the difference, if any, between the spontaneous act of clotting or setting, e.g. of blood recently shed, of milk to which rennet has been added, and the separation by heat of proteid bodies, we do not propose to enter at all fully; and whether Gerinnung be one thing and Abscheidung another, this we must leave undecided. Neither have we in the first instance attempted to determine what might be the essentials, if one may use the expression, of the act of heat coagulation, we have simply taken that act and noted its modification by the presence of certain salts in certain proportions, and in respect of this same act we have examined the mutual reaction of these salts upon one another when present together. Secondly, we have touched upon the question of the essential factors of heat coagulation, but this really represents a side issue.

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[1] This *Journal*, Vol. xi. p. 369.

The liquid with which we have chiefly worked has been serum, and in the majority of cases it has been obtained from sheep's blood. In general this serum has been diluted with twice its volume of distilled water.

Dr Halliburton[1] has devoted much attention to the question of the proteids of serum, and he has shewn that, besides the main division of these bodies into serum globulin and serum albumin, the albumin of serum may, by fractional heat coagulation, be analyzed into varieties to which he has provisionally assigned the symbols a, β, γ. Further Dr Halliburton has tested the influence of salts upon the separation of these albumins, more particularly by the method of double saturation. From his experiments it would appear that the reaction of the serum is of cardinal importance in testing the temperature levels of heat coagulation, up to a certain point coagulation being facilitated by increased acidity.

Limburg[2] has stated in an investigation into the influence of salts upon the solution and precipitation of proteids that the separation temperatures both of albumins and globulins are modified by the presence of salts. He says "it would appear that by alteration of the saline percentage the coagulation temperature of a proteid solution is within certain limits first raised and then gradually lowered, till in the end the atmospheric temperature may alone suffice to separate the proteid." "Globulins," he further says, "differ from albumins in that they (the former) shew, for the most part, a lowering of their coagulation temperature in the presence of salts, whilst albumins shew the reverse." This is given as a broad statement.

In our experiments we have tested the action of the salts of lime, strontium, barium and magnesium, and likewise that of sodium and potassium chloride. We have also sought to determine the effect upon one another, in respect of heat coagulation, of salts taken from these two groups.

Our method has been to dilute a portion of serum which had separated by spontaneous coagulation, to add to this diluted serum the salts under investigation, and then to raise the temperature of such modified solutions by means of a water-bath. The temperature at which the proteid has begun to separate out has then been noted. Each experiment has been controlled by subjecting to the same conditions a portion of the diluted but otherwise unmodified serum.

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[1] This *Journal*, 1884—1885.

[2] *Zeitschr. f. physiol. Chemie*, XIII. 450. 1889.

The following experiments were made:—

Experiment I. *Oct.* 18. Serum from sheep's blood shed on Oct. 15, diluted by the addition of two volumes of distilled water. Of this fluid 10 c.c. were placed in each of six test tubes. One test tube was used as control to the other five, the following quantities of a 10 °/$_0$ solution of *calcium chloride* were added, viz., 1, 2, 4, 6 and 12 drops respectively. In the results these numbers will represent the several test tubes.

The test tubes were then placed in a water-bath the temperature of which was gradually raised.

At 58° C. 4 and 6 became slightly turbid.
61° ,, They were markedly turbid.
63° ,, 12 was distinctly turbid.
65° ,, 4, 6, 12 were completely opaque.
2 was opalescent.
69° ,, 4, 6, 12 were semisolid.
2 more opaque, 1 slightly turbid as compared with the control.
72° ,, 2 nearly as opaque as 4, 6, 12 but much more fluid.
1 distinctly opaque.
74° ,, 4, 6, 12 more solid; 2 semisolid; 1 more opaque.
78° ,, Control slightly turbid.
85° ,, 4, 6, 12 solid, firm; 2 solid; 1 opaque and thick; control opalescent.
91° ,, 1 had set; control more opalescent but quite fluid.

The order of separation in this experiment was therefore in descending sequence, beginning with the higher percentages of calcium chloride solution. The test tubes 4 and 6 came out a little earlier than the 12; this however was probably accidental as the difference was but slight.

[In this experiment the drops were from the bottle and were larger than those of preceding experiments. In all subsequent experiments the drops were from a pipette and were uniform in size. They were smaller than the above.]

Experiment II. In another experiment made on *Oct.* 18, with a *nitrate of lime* solution 10 °/$_0$. The quantities of Experiment I. were repeated. The results obtained were as follows:—

65° C. 4, 6, 12 turbidity commencing, but most marked in 12.
70° ,, 12 quite opaque; 4 and 6 turbid.
71° ,, 12 densely opaque, semisolid; 6 more opaque than 4.
75° ,, 6 very opaque, 4 less so; 2 faintly opaque.
78° ,, 4 quite opaque but less than 6; 2 distinct opacity.
79° ,, 6 semisolid; 4 thick.

80° C. 12 quite solid; 6 also.
81° „ 4 solid.
86° „ 2, 1 and control opaque or opalescent; no great difference between them.
97° „ No difference between these three.

In this experiment the order of separation was strictly in accordance with the proportion of lime present. The drops in this experiment being smaller than those of Experiment I. we might expect a slight delay in the first appearances of turbidity.

In an experiment made on *Oct.* 16 with *calcium chloride*, a 10 °/₀ solution, the results obtained were similar to those recorded. The tubes containing 4, 6 and 12 drops did not however shew any difference in either the rate or the amount of heat coagulation.

In an experiment with calcium nitrate, a 10 °/₀ solution, made on *Oct.* 25, the sheep's serum was from blood shed on *Oct.* 22. With this Experiment II. was repeated; the results obtained were very similar to those recorded, viz., 12 slightly preceded 4 and 6 at the temperatures 62°—65°; at 78° all three were solid. Test tube 2 did not begin to become opaque till 68°, it became solid at 82°. Test tube 1 began at about 68°—73° and at 95°—97° became a soft jelly. The control became opalescent at 77°, milky at 81°, thick but quite liquid at 97°.

The differences in the last experiment from the results of Experiment II. are probably due to differences in the quality of the serum.

On *Oct.* 28 working with undiluted serum the effect of lime in favouring heat coagulation was quite distinct though the limits of divergence were much nearer together, e.g. the control became solid at 77° C., and the presence of 12 drops of $CaCl_2$ solution (10 °/₀) were only able to lower this temperature 6 degrees, for the 12 tube became solid at 71° C. (Undiluted serum is of course much more coagulable than diluted serum.)

It will not be necessary to give further evidence of the favouring influence of salts of lime upon the process of heat coagulation of the proteids of serum, for the results are quite uniform, and the same evidence will be again forthcoming in experiments which shew the comparative or antagonistic action of salts.

Experiment III. In this, serum from sheep's blood was diluted with two parts of distilled water, and with this the Experiments I. and II. were repeated with the substitution of *strontium chloride* (a 10 °/₀ solution) for the calcium salt.

Six test tubes were taken, each containing 10 c.c. of the diluted serum.

Five of these received strontium chloride in the following quantities, viz., 1, 2, 4, 6 and 12 drops respectively; the 6th test tube remained as control. The test tubes were then placed in a water-bath the temperature of which was gradually raised.

Oct. 24. At 65° C. 12 became slightly turbid; this increased rapidly.
69° ,, 2, 4, 6 were slightly hazy.
70° ,, 12 shewed a dense white opacity.
72° ,, 2, 4, 6 opalescent.
74° ,, 2, 4, 6 milky, 1 hazy (73°).
75° ,, 12 thick.
79° ,, 2, 4, 6 white but 6 was most opaque.
80° ,, 12, 6 solid.
81° ,, 4 gelatinous, 2 thick, 1 milky, control opalescent.
82° ,, 4 solid.
97° ,, 2 semisolid, 1 still quite liquid, control more opalescent.

The order of coagulation was here also in proportion to the quantity of strontium chloride, and on comparing this experiment with the lime experiments it will be seen that the strontium chloride effect corresponds closely with the calcium nitrate effect. It falls short of the calcium chloride effect (Experiment I.), but in that experiment, as mentioned, the drops of the lime solution were of larger size.

In a previous experiment with strontium chloride made on *Oct.* 18 results were obtained quite parallel with those of Experiment III. Tube 12 led off at 65°, was closely followed by tube 6, whilst both became solid at 75°. Tube 4 began to be cloudy at 72°, at 80 became solid. Tube 2 became cloudy at 75°, became solid at 89°. Tube 1 became cloudy at 80°, but remained liquid at 97°.

Slight differences in the quality of the serum will probably explain the slight differences in these two experiments with strontium chloride, but both point to a very decided favouring influence of the strontium salt upon heat coagulation.

Barium chloride.

EXPERIMENT IV. Substitution of *barium chloride* (10 $^0/_0$) for the lime and strontium salts.

Oct. 20. The serum used was from blood shed on Oct. 15, it had a faint, rather offensive odour; it was diluted as usual.

At 67° C. 12 became turbid.
68·5° ,, 4, 6 turbid.

70° C. 12 densely opaque, 2 turbid, 1 opalescent.
73° ,, 12 semisolid (control faintly opalescent at 72°).
75° ,, 2, 4, 6 milky (2 rather less than 4 and 6).
80° ,, 12 solid, 4 and 6 gelatinous.
82° ,, 4 and 6 solid.
83° ,, 2 semisolid, 1 milky, control opalescent-milky.
88° ,, 1 a little thick.
94° ,, 2 solid.
95° ,, 1 semisolid.
96° ,, Control milky but quite liquid.
97° ,, 1 rather more solid, control quite fluid.

Again, these results shew a close similarity of effect between the action of barium chloride and that of strontium and calcium chloride. Barium however falls a little short of both of these salts.

A previous experiment with barium chloride on *Oct.* 18 gave parallel results to those of *Oct.* 20, but the BaCl$_2$ in this was rather more active.

Sulphate of magnesium.

Experiment V. A 10 $^{0}/_{0}$ solution of *magnesium sulphate* was added to the diluted serum (sheep's blood) in the same quantities as before. The test tubes are named according to the number of drops of the magnesium solution they contained.

Oct. 24. The serum was from blood shed on Oct. 22.
At 69° C. 12 shewed a haziness.
73° ,, 12 was milky, 2, 4, 6 opalescent.
75° ,, 12 densely white, 1 opalescent.
76° ,, 6 milky.
77° ,, 2, 4 milky.
78° ,, 1 more turbid, but less than 2 and 4, control hazy.
79° ,, 12 solid, 6 thick.
80° ,, 6 semisolid.
81° ,, 4 densely white, solution thick.
83° ,, 6 solid, 4 nearly solid.
85° ,, 4 solid, 1 milky.
92° ,, 2 semisolid.
93° ,, 2 gelatinous.
97° ,, 2 as above; 1 ? a little thick; control turbid.

These results shew that magnesium sulphate acts similarly to the salts of the calcium group. In degree of action it differs but slightly from them.

A previous experiment on *Oct.* 19 with serum some 4—5 days old gave results parallel to the above, but the salt in this case appeared rather more effective, for tube 12 became solid at 77°, and even tube 1 became semisolid at 86°. In this case the control itself became more turbid or milky than in Experiment V., so that the difference in the serum, whether primary or as the result of keeping, must account in part for this difference observed.

EXPERIMENT VI. In this serum, diluted with two parts of distilled water, was placed in 4 test tubes (in each 10 c.c.) To the 4 were added respectively

2 drops of $CaCl_2$ 10 %. A.
" " $SrCl_2$ " B.
" " $BaCl_2$ " C.
" " $MgSO_4$ " D.

Clouding began in A at 70° C.
" " B " 73° "
" " C " 74° "
" " D " 77° "

So far as this one experiment goes it points to a greater activity of calcium. The difference between B and C is inappreciable. No conclusion however can be drawn here, nor do the experiments detailed above bear out such difference.

Sulphate of zinc.

Oct. 31. One experiment, No. VII., was made with this salt (a 10 % solution) on account of the resemblance which it bears to magnesium salts, e.g. in the isomorphism of the salts of magnesium and of zinc. Four test tubes were taken, each received 10 c.c. of diluted serum. The 1st remained as control, the 2nd received 2 drops of the *zinc* solution, the 3rd 4 drops, the 4th 6 drops.

All the tubes behaved exactly alike; they became a little cloudy between 78° C. and 80°; milky between 78° and 85°. They were all quite liquid at 95°.

On *Nov.* 1, after standing 24 hours the three zinc sulphate tubes 2nd, 3rd, and 4th, in this order received 2, 4 and 6 drops respectively of calcium chloride (10 % solution). They were then placed in a water-bath and the temperature raised.

The control did not clot, the three lime tubes did, though somewhat irregularly; thus the 2nd tube containing 2 drops of zinc sulphate solution + 2 drops of $CaCl_2$ became solid at 90°, whilst the 3rd and 4th tubes shewed only partial clotting. Hence whilst we cannot compare amongst themselves the lime tubes we again get evidence of the positive influence of lime in aiding heat coagulation.

The next point of investigation concerned the mutual antagonism of salts in respect of heat coagulation. The salts chosen were the chlorides of sodium and potassium.

Experiment VIII. On *Oct.* 27 six test tubes received each 10 c.c. of serum of sheep's blood diluted with twice its volume of distilled water. The blood had been shed on Oct. 22, and the serum was slightly offensive. The control test tube received 3 drops of $CaCl_2$ (10 °/₀ solution). Each of the remaining tubes received the same quantity of lime chloride and the following quantities of NaCl (a 10 °/₀ solution), viz. 0·5 c.c., 1 c.c., 1·5 c.c., 2 c.c. and 3 c.c.

At 64° C. the control began to cloud; at 69° it was milky; at 75° a soft solid.

At 70° C. the 0·5 c.c., 1 c.c., 1·5 c.c., and 2 c.c. NaCl began to cloud; at 72° they were milky; at 77° the 0·5 c.c. and 1 c.c. were coagulated into a soft solid; at 79° the 1·5 c.c. shewed the like; at 79—80° the 2 c.c. shewed the like.

At 72° C. the 3 c.c. NaCl began to cloud; at 80° it was slightly solid.

In this experiment the tubes were unequally diluted, according to the amount of NaCl solution added, and accordingly on the same day and with the same serum the following experiment was made. Two test tubes containing each 10 c.c. of diluted serum received

A. 3 drops of $CaCl_2$ + 4 c.c. of distilled water.
B. 3 drops of $CaCl_2$ + 4 c.c. of a 10 °/₀ solution of NaCl.

At 65° C. A began to cloud; at 70° it was turbid; at 74° densely turbid; at 81° semisolid.

At 71°—72° B began to cloud; at 75° was turbid; at 79° opaque; at 82° densely opaque; at 85° semisolid.

In this experiment there was a distinct controlling of the coagulation by the NaCl, and this was also quite distinct in Experiment VIII. in the case of the tube with the 3 c.c. of NaCl.

In preceding experiments on *Oct.* 17 and *Oct.* 25 there was observed the same effect of NaCl in delaying heat coagulation in the presence of calcium chloride.

On *Oct.* 17, 10 c.c. of serum + 1 c.c. of distilled water + 3 drops of $CaCl_2$ 10 °/₀ solution began to cloud at 60°, at 75° became very opaque, at 78° semisolid. Compared with this the same quantity of serum and of lime+1 c.c. of a 10°/₀ solution of NaCl in place of the distilled water did not begin to cloud till 75°, and did not become semisolid till 82°.

On *Oct.* 25, taking only the extreme terms of the experiment, it was found that:

10 c.c. of serum + 2 drops of $CaCl_2$ solution + 2 c.c. Aq. began to cloud at 70°.

10 c.c. of serum + 2 drops of $CaCl_2$ solution + 2 c.c. NaCl solution did not begin till 77°.

The coagulation once started proceeded rapidly in all the test tubes (there were four), but it took place in the order of less NaCl to more NaCl.

Sodium chloride controls therefore the action of a lime salt ($CaCl_2$) to some slight extent.

With regard to potassium chloride by itself, several experiments with diluted serum—1 in 10—shewed that the potassium salt behaved quite like the sodium salt in respect of heat coagulation; this will be referred to later. In respect of its restraining influence upon lime chloride it appeared to act like sodium chloride. An experiment on this point was made in November, the results obtained however were not striking. Though this point has not been thoroughly tested, sufficient has been done to make it probable that NaCl and KCl run very parallel in respect of their influence upon lime.

Whilst however NaCl and probably also KCl restrain to some extent the action of calcium chloride, they by themselves appear to favour heat coagulation; i.e. the addition of either salt to a specimen of serum facilitates the separation of the proteids by heat. This is best tested with fresh serum having a neutral or faintly alkaline reaction.

EXPERIMENT IX. In two such experiments made on *Nov.* 11 and 12, with quantities of potassium and sodium chloride varying from 0·2 c.c. to 2 c.c. of 10°/₀ solutions—these quantities being added to 10 c.c. of the serum—it was noted that heat coagulation first appeared in the test tubes containing the highest amounts of the salines; this occurred at the temperatures of 69°, 70°. The controls on the contrary did not give any clouding till from 77°—78°.

At the end of the experiment, at temperatures of 95°—96° the amount of coagulation as measured by the degree of turbidity presented an ascending scale from the control up to the tubes containing 1 c.c. and 2 c.c. of salines.

In the KCl experiment on *Nov.* 12 the tubes were next allowed to stand 24 hours, then filtered, then rendered acid with 1 drop of a 27 °/₀ solution of acetic acid to each tube. On reheating this solution an abundant precipitate occurred in all, but now in descending scale from the control to the highest saline. This confirmed the previous results.

The reason for testing the point with fresh serum is that the acidity which develops on prolonged keeping of serum, e.g. on dialysing it, causes the more complete precipitation of the proteids, and the moderate effect of KCl and NaCl is unrecognizable.

These experiments suggest that sodium and potassium chlorides whilst favouring to some extent heat coagulation, interfere somewhat with the activity of calcium chloride, though this activity is in the same direction. Such interference of similars is interesting. Calcium salts are however far more powerful than sodium or potassium chloride in favouring heat coagulation.

The next point for determination was the part played by lime in heat coagulation. Was it essential? Could a proteid free from lime coagulate under any circumstances? This question we have not been able to solve for the reason that we have been unable to obtain a lime-free proteid. For even though by dialysis of serum or by the dissolving of washed globulin in sodium chloride solution we have obtained solutions of proteids which gave either no reaction with ammonium oxalate or an indefinite one, yet the same solutions when coagulated, the coagulum collected and incinerated, yielded an ash which on treatment with hydrochloric acid and neutralization with ammonia and further addition of free acetic acid gave a turbidity with ammonium oxalate.

In the case of globulin, to give one experiment, our method was as follows:—

EXPERIMENT X. 150 c.c. of blood were poured into 7000 c.c. of distilled water. A precipitate of globulin took place which gradually subsided. At the end of two days the supernatant fluid was syphoned off and then the layer of liquid containing the suspended globulin was filtered. The residue on the filter-paper was washed with distilled water, the washings giving no lime reaction with ammonium oxalate. The washed globulin was then dissolved in a 0·6 °/₀ solution of sodium chloride.

This solution of globulin coagulated on heating. The addition of calcium chloride solution to a fresh portion of this globulin solution did not give any increase of coagulation on heating.

Whereas however before coagulation the globulin solution gave no reaction with ammonium oxalate, it was found that after coagulation and removal of the coagulum by filtration the filtrate did give a slight lime reaction.

The conclusion is that lime in minute quantity attaches itself to the proteid molecule so closely that by diffusion it cannot be removed. In the act of heat coagulation it would appear from the last experiment that dissociation of the lime takes place. The nature of the union it is impossible to speculate upon but, in the case of globulin, it is probably more intimate than a mechanical carrying down would express.

Thus far then the question as to the essential presence of lime in the act of heat coagulation remains unsolved, for we have been unable to remove the last traces of lime from the proteids.

It remains to ask what is the nature of the favouring influence of lime on the process of heat coagulation of which we have spoken. The quantities of lime here employed have been very appreciable ones, viz. from 2—12 drops of a 10°/₀ solution of the lime salt, for every 10 c.c. of the serum, diluted or undiluted. It is more than probable that these quantities of lime, even the lower numbers, are far in excess of those actually required in the chemical changes which the proteid undergoes in coagulation, that in other words the lime is not wholly used up and carried down as an insoluble compound with the proteid. If this be so then the influence of such superabundant lime must be of the nature of a removal of restraint, the lime inhibiting a restraining cause. In evidence of this we have some experiments which shew that if a solution of serum be rendered alkaline with caustic soda the proteid is almost wholly prevented from coagulating—such fluid being less affected by heat than the simple diluted serum—but that by the addition of calcium chloride (a neutral salt) the restraining influence of the caustic soda may be completely overcome.

EXPERIMENT XI. *Monday, Oct.* 20. Serum from blood (sheep's) shed on Oct. 15, and having a faint, slightly offensive odour, diluted with two volumes of distilled water. To 100 c.c. of this serum 2 c.c. of a 1 °/₀ solution of NaHO were added. This solution, of which the alkalinity was 0·0002 °/₀, was strongly alkaline to test-paper. Six test tubes were taken.

a = a control of dilute serum without NaHO.
b = control ,, ,, with NaHO.
c = alkalinized serum + 1 drop of $CaCl_2$ 10 °/₀.
d = ,, ,, + 3 drops ,, ,,
e = ,, ,, + 6 drops ,, ,,
f = ,, ,, + 12 drops ,, ,,

At 65° C. precipitate began to form in *f*.
69° ,, *f*, precipitate dense white.
70° ,, *e* and *d* faint opalescence.
72° ,, *a*, faint opalescence.
75° ,, *f*, semisolid; *e*, milky; *d*, opalescent; *a*, opalescent.
80° ,, *b* and *c* faint opalescence.
82° ,, *f* = solid.
85° ,, *e* = solid.
90° ,, *d*, milky but liquid; *a*, milky but liquid.
97° ,, *d*, ? a little thick; *a*, no change.

Here, coagulation occurred first in the tube containing most calcium chloride, it then followed in descending order in the tubes containing less and less calcium chloride, but in the tube containing only 1 drop of calcium chloride coagulation appeared rather later than in the non-alkalinized control. This must mean that the alkali was more than sufficient to be overcome by the small quantity of lime salt.

The last-mentioned experiment was repeated on the same day and with the same result, only more strikingly shewn. In this experiment the quantity of sodium hydrate was doubled, viz. 4 c.c. of a 1 $^0/_0$ solution to 100 c.c. of diluted serum, and here even 3 drops of calcium chloride solution failed to overcome the alkalinity, but 6 and 12 drops proved effectual and the tubes became solid at temperatures 84° and 78° respectively.

An interesting modification of this experiment was made by alkalinizing the serum by means of lime water and then adding calcium chloride solution.

Experiments of this kind were made both with ordinary serum, diluted with two volumes of lime water, and also with dialyzed serum, the acidity of which was overcome by adding 18 drops of lime water to 10 c.c. In either case the solution was very distinctly alkaline. Such alkalinized serum was decidedly less coagulable than serum diluted with water only, but by the addition of calcium chloride the restraint of the alkalinity was overcome and the solutions became solid on heating. The results were very definite. Another experiment with a solution of globulin was made and with the same results.

The value of this experiment with CaH_2O_2 is that on the addition of $CaCl_2$ no double decomposition can occur with the formation of other bodies, as may happen in the case of caustic soda or carbonate of soda. So far as can be seen the sole restraining influence of lime water is its alkalinity, and this is overcome by a neutral salt of lime.

Experiments were also made with serum acidulated with acetic acid. As is well known and as is expressly mentioned by Halliburton[1] the degree of acidity of albuminous solutions is very important in the study of heat coagulation. Beyond a certain degree of acidity coagulation is not favoured but retarded, and it may be completely restrained. Calcium chloride added to solutions whose coagulability is partially restrained will in part remove that restraint, the solution again becoming coagulable by heat. Experiments of this kind were made in October with diluted serum and also with solutions of desiccated blood.

To recapitulate briefly. Lime and the allied elements favour the heat coagulation of the proteids of serum. A similar action is exerted by magnesium sulphate. Magnesium has affinities with the calcium, strontium and barium group, and it also in some respects approaches zinc, but the resemblance to zinc is probably of a different kind, for we have not found that zinc possesses any favouring influence on clotting. The action of lime has been tried with two of its salts, the chloride and nitrate, and it probably is a class reaction; the like probably holds for strontium, barium and magnesium, though we do not furnish experiments in proof of this. Salts from this group of bodies being many of them insoluble, it is difficult to investigate the subject very exhaustively.

It is of interest to note that in the process of coagulation by heat, as in the process of spontaneous clotting of blood and the clotting of milk to which rennet has been added, lime plays an important part. This similarity of behaviour holds also for barium and strontium as will be seen in our paper on the influence of salts on the act of clotting[2]. In this paper we gave reasons for regarding the action of lime, strontium and barium as generic, and it is for this reason amongst others that we are inclined to believe that the influence of these same elements upon heat coagulation will be found to belong to their salts generally. One is led to infer a possible likeness in nature between heat coagulation and spontaneous clotting of proteid bodies. We learn from Dr Halliburton that very little is known about the chemical changes which take place when albumin is coagulated by heat, but that the general assumption is that the percentage composition of the albumin remains unaltered. More than this cannot be stated, so he says, till the nature of the proteid molecule has been revealed to us. In the case of fibrinogen the case is somewhat different for Dr Halliburton informs us that

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[1] This *Journal, loc. cit.*

[2] *Ibid. loc. cit.*

Hammarsten has shewn that when it is coagulated by heat a proteid split off from the fibrinogen molecule goes into solution[1].

That there are differences between heat coagulation and spontaneous clotting we learn from one fact in particular, viz. that in the former the solution becomes more alkaline, whereas in the latter, as also in the stiffening of muscle in rigor mortis, there is a development of acid[2].

As matters stand then we would only insist on the similarity which obtains both for heat coagulation and spontaneous clotting in respect of the influence of the elements of the alkaline earth group upon these processes.

Next, with regard to the presence of a lime as an essential factor of heat coagulation, this question we leave unsolved, since we have been unable to remove the last traces of lime. As the result of our investigation however, we suggest that the favouring influence of lime salts when employed in appreciable quantities is of the nature of a removal of restraint. This we have illustrated more particularly by the action of lime in removing the restraint of an excess of alkali or acid.

We have further shewn that between potassium and sodium chloride on the one hand, and lime chloride on the other, a certain amount of antagonism exists. In this respect our results were similar to those obtained with these same salts when we were investigating spontaneous clotting. With this difference, however, viz. that sodium and potassium chloride, when used singly, appeared to have some slight favouring action upon the separation of the proteids by heats. This subject we did not follow out, and further experiment is necessary to establish the fact and the limitations of this statement; more especially in respect of the all important question of dose it would be necessary to extend these last experiments.

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[1] *Pflüger's Archiv*, xxii. 480.

[2] Halliburton. "Proteids of Serum." This *Journal*, Vol. v. p. 155.

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[*From the Journal of Physiology. Vol. XIII. Nos.* 3 *&* 4, 1892.]

FURTHER OBSERVATION ON THE INFLUENCE OF CALCIUM SALTS IN PROMOTING HEAT COAGULATION OF ALBUMINS. By SYDNEY RINGER, M.D., F.R.S.

(From the Physiological Laboratory of University College, London.)

In previous communications in this Journal I have drawn attention to the influence of calcium salts on the coagulation of alkali albumin. In this communication I treat of the influence of calcium salts on a native albumin, namely, white of egg.

A weak solution of egg albumin in distilled water (1 in 8) heated slowly to boiling point does not coagulate, but if a small quantity of calcium chloride is first added, the solution coagulates readily. This might at first sight be considered a proof that a calcium salt greatly favours coagulation of egg albumin. This however is not the case. On heating the dilute solution without the addition of a calcium salt the albumin is converted into alkali albumin, and this is readily coagulated on the addition of a soluble calcium salt; and I submit that the coagulation occurring during the heating of dilute egg albumin, to which calcium chloride is added, is due to the albumin being first converted into alkali albumin and then precipitated by the action of the calcium salt.

The following experiments[1] show that if dilute white of egg is made faintly acid with acetic acid, and thus its conversion into alkali albumin by heat prevented, it coagulates without the addition of a calcium salt; and the addition of calcium chloride neither accelerates the coagulation nor increases the amount coagulated in slowly raising the temperature to boiling point.

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[1] In the experiments recorded in this paper I used a 10 $^0/_0$ neutral solution of calcium chloride and in some a 10 $^0/_0$ solution of calcium nitrate. I dropped the solution from a pipette that gave fourteen drops to the c.c.

I diluted white of egg with distilled water, 1 in 8, and filtered through blotting-paper, and then added 3 drops of 10% solution of acetic acid to 30 c.c. of the albumin mixture. The mixture became a little opalescent, and was acid in reaction. Three test-tubes, the second containing 3 drops, the third 6 drops of calcium chloride solution, received 10 c.c. of the acidulated albumin solution. The calcium chloride removed the opalescence in the tube with 6 drops and reduced it in the tube with 3 drops. The test-tubes were then placed in a water bath and the temperature raised. Each of the solutions became cloudy at 59, and the solution without calcium chloride became particulate at 59° C.; the solution with 3 drops at 59·5° C.; and the solution with 6 drops at 60° C. After boiling they each set into a loose clot. I then filtered each coagulated solution and saturated the filtrate with ammonium sulphate. There occurred a slight deposit in each solution and about the same in quantity in each tube.

I next repeated this experiment with a solution of 30 c.c. of albumin mixture which received only 2 drops of acetic acid 10% solution. This gave a faint acid reaction. The solution in each of the tubes became cloudy and particulate at 59° C. On filtering and treating the filtrate with ammonium sulphate, the amount of albumin precipitation by boiling appeared to be the same in each tube, as the ammonium sulphate produced only a slight precipitate, judging by the eye, equal in amount in each tube.

I repeated the experiment adding 1 drop of the acetic acid solution to 30 c.c. This solution gave a faint acid reaction, the litmus becoming about as red as the redness produced by distilled water. The solution without calcium chloride and the solution with 3 drops of the calcium solution became opalescent at 56° C.; the solution with 6 drops at 57° C. The first two solutions became particulate at 57° C. and the third at 60° C. On boiling, the solution in the first tube became viscid and did not set; the fluid in the other tubes set into a loose clot.

In some experiments with the smallest quantity of acetic acid the influence of boiling on the solution without calcium chloride differed. In some it became gelatinous, and filtered through blotting-paper very slowly, but the filtrate when saturated with ammonium sulphate only precipitated a small quantity of proteid, no greater than in the filtrate from the solutions containing 3 and 6 drops of calcium chloride solution. The calcium chloride therefore in this experiment did not influence the precipitation of the proteid, but assisted the aggregation of the particles. In another experiment however, with albumin from another egg, the solution without calcium chloride only became cloudy, and on testing it I found it changed into alkali albumin.

In the previous experiments the addition of calcium chloride appeared to have no influence on coagulation of egg albumin by heat. Still these experiments do not prove that calcium salts are without influence on heat coagulation, for the calcium salt present in the native albumin may suffice with the aid of an acid, and the further addition of a calcium salt may not increase the action of the calcium salt naturally present. I made the following experiments to get rid of the soluble calcium salts in egg albumin.

I diluted the white of an egg with distilled water and precipitated the albumin by saturation with ammonium sulphate, filtered and redissolved in 200 c.c. of distilled water: I repeated the precipitation and solution three times. I then performed the following experiments. The first test-tube contained 10 c.c. of the dissolved albumin; the second 10 c.c. with 3 drops of calcium chloride solution, and the third tube 10 c.c. with 6 drops of calcium chloride solution. Placed in a water bath and slowly heated, at 69° C. the fluid in each of the tubes became opalescent, at 76° C. milky, and at 80 particulate. On boiling, all the solutions became semisolid.

It is true on the addition of ammonium sulphate the calcium would become converted into the slightly soluble sulphate, but the solution in a large quantity of distilled water and filtration I think must have removed most of the calcium from the egg albumin; and yet the solution to which no calcium chloride was added coagulated at the same temperature as the solutions to which calcium chloride was added.

I next performed similar experiments, but first added to one specimen weak hydrochloric acid, and to another some acetic acid, thinking that these acids would withdraw any calcium that might be combined with the proteid. I then precipitated with ammonium sulphate, filtered and dissolved. I give in detail the experiment with acetic acid.

I diluted 35 c.c. of white of egg to 200 c.c. with distilled water and filtered through muslin; I then added 25 c.c. of 10°/₀ solution of acetic acid to remove any calcium that might be combined with the proteid; I next precipitated the albumin by ammonium sulphate and then filtered. The albumin dissolved readily in 75 c.c. distilled water and gave a strong acid reaction. It coagulated readily on boiling. I reprecipitated with ammonium sulphate and again filtered and dissolved the albumin in 300 c.c. distilled water. The solution gave a strong acid reaction. A little boiled coagulated readily. I again precipitated the albumin by ammonium sulphate and again filtered. I dissolved the precipitated albumin in 200 c.c. of distilled water. The solution was decidedly acid in reaction. A little quickly boiled coagulated abundantly. I then filtered the dissolved albumin, and performed six experiments, in each employing 10 c.c. of the solution. To the first tube I added no calcium chloride; to the second test-tube I added 1 drop of calcium chloride solution; to the third 2; to the fourth, 3; to the fifth, 4; and to the sixth, 6 drops. After five hours there was no change in the solutions. I then placed the test-tubes in a water bath and slowly raised the temperature. Each of the solutions became particulate between 55° C. and 57° C.; and when boiled the albumin clotted, the amount of deposit, judging by the eye, being the same in each tube.

I incinerated some of the coagulated albumin to which no calcium chloride had been added, dissolved in strong hydrochloric acid, neutralized with ammonia and made the solution slightly acid with acetic acid. The addition of ammonium oxalate precipitated a small quantity of calcium oxalate. Some calcium salt therefore still remained in the solution, but a great part must have been washed away, and yet the albumin coagulated with heat; and calcium chloride had no influence in accelerating or increasing the coagulation.

The experiments with hydrochloric acid gave the same results. All the calcium was not removed, as the ash yielded a minute trace of a calcium salt.

I next attempted to get rid of all calcium salts from egg albumin by the action of oxalate of ammonium. To white of egg diluted with seven parts of distilled water I added ammonium oxalate in excess, and after six hours decanted the supernatant fluid and filtered. This solution I rendered slightly acid by the addition of 3 drops of 10 °/₀ solution of acetic acid to 30 c.c. The solution to which no calcium chloride was added coagulated by heat, the addition of calcium chloride to the other specimens not accelerating nor increasing the coagulation.

I next dialysed a mixture of egg albumin (1 in 8), adding 15 c.c. of 10 °/₀ solution of acetic acid to 100 c.c. of the albumin mixture. The dialyser was placed in 1000 c.c. of distilled water for seven days. The water was first renewed twice and then once daily. At the end of this period the fluid gave a slight acid reaction, about as marked as that of distilled water. The solution had not decomposed. It was quite sweet to the smell.

I made many observations, each consisting of two experiments; 10 c.c. without any calcium salt added, whilst the other 10 c.c. received 6 drops of calcium chloride solution. The test tubes were then placed in a water bath and the temperature slowly raised. The solution without calcium chloride became cloudy between 55° C. and 56° C. and particulate at 61° C. to 62° C. The solution containing calcium chloride became cloudy at 68° C. to 69° C.

and particulate at 73° C. to 74° C. Hence the addition of calcium chloride retarded coagulation.

After boiling, the solutions in each tube set into a clot, and on filtering and testing the filtrate by saturation with ammonium sulphate I found that the amount of proteid deposited was the same in each tube.

Although the acetic acid dialysed out and also most of the salts, for the albumin mixture contained no chloride, yet after this long interval some calcium remained. I evaporated 30 c.c. to dryness, incinerated in a platinum crucible, and then added a drop of strong hydrochloric acid and next diluted with distilled water, and made the solution slightly alkaline with ammonia and next slightly acid with acetic acid. I then added oxalate of ammonium, and after standing for some time the solution became faintly opalescent and a very slight deposit occurred. The calcium salt was probably combined with the proteid, for the addition of ammonium oxalate to the albumin mixture caused no precipitate, and, after boiling and coagulating, some of the clear filtrate did not precipitate on the addition of ammonium oxalate. The incinerated coagulated albumin however gave a calcium reaction.

These experiments I submit prove that calcium chloride and calcium nitrate, and probably other soluble calcium salts, have no influence on the heat coagulation of egg albumin unless it is first converted into alkali albumin.

How do the soluble calcium salts precipitate alkali albumin from its solutions?

It is held in solution by an alkali. In most of my experiments by sodium bicarbonate.

If the alkalinity is removed by any means, then the proteid is deposited. This of course occurs on the addition of an acid. The suggestion therefore at once arises that calcium chloride solution acts in this way, and many of the reactions favour this view. When calcium chloride is added to a solution of sodium bicarbonate, a precipitate at once occurs: except with weak solutions, the precipitate of calcium carbonate takes a little time. Heat accelerates the precipitation of calcium carbonate with weak solutions; and if the solutions are very weak, precipitation only occurs with the aid of heat.

Now this is exactly what happens on adding calcium salts to alkali albumin solution. If a large quantity of the calcium salt is added, precipitation occurs at once at the temperature of the room; if a small quantity is used, then a little time is required before precipitation occurs. With small quantities of calcium salt, heat greatly accelerates the precipitation, and with very small quantities of the calcium salt, precipitation of the proteid only occurs with the aid of heat.

There can be no doubt therefore that calcium salts act in some instances by lessening alkalinity and so destroying the solvent action of the menstruum. But is this the whole explanation of their action, or have calcium salts an additional property whereby they precipitate alkali albumin?

There can I think be no doubt that calcium salts are able to precipitate alkali albumin by some property other than that of diminishing or neutralizing the alkalinity of the solution.

If calcium salts precipitate alkali albumin simply by neutralizing alkalinity, the weak acids should act in the same way as calcium salts. The following experiments show that weak hydrochloric acid must be added to these solutions of alkali albumin till the solution is decidedly acid, before any precipitation occurs even at a boiling temperature. Now, a small quantity of calcium nitrate or calcium chloride (neutral) solution will precipitate alkali albumin from alkaline solutions. Further, the precipitate produced by the addition of an acid even when boiled dissolves on the addition of a little caustic soda solution, whilst the precipitate with a calcium salt is unsoluble.

I prepared alkali albumin by diluting white of egg with 7 parts of distilled water and slowly heating to boiling point. This preparation is not precipitated till the solution has a decided acid reaction. As the point of precipitation is being reached the fluid becomes a little milky, but still not particulate, and will filter through blotting-paper without any deposit on the paper.

Then a very slight further addition as of one drop of ·2 $^0/_0$ HCl to 10 c.c. of the alkali albumin solution causes abundant precipitate and almost the whole of the proteid is deposited. If the solutions just short of precipitation are heated, precipitation occurs; but other solutions containing only a very little less acid, and still acid in reaction, will not precipitate nor become particulate on boiling.

Four test-tubes received 10 c.c. each of albumin mixture. Into the first I dropped slowly 36 drops of ·2 $^0/_0$ HCl; into the second 37; into the third 38 and into the fourth 39. Forty drops caused an abundant precipitate. Each of the solutions gave an acid reaction. The first two solutions were scarcely changed, becoming only a little opalescent; the third became a little, and the fourth still more milky. None were particulate. The solutions stood 24 hours and at the end of that time the cloudiness was a little settling, the fluid being clear at the top; but none of the solutions were particulate to the naked eye. I then slowly raised the temperature to boiling. The two solutions with the larger quantities of acid became particulate at 56° C. The solution with 37 drops became particulate on boiling, whilst the solution with 36 drops was unaffected. Nearly the whole of the proteid was precipitated in the three tubes with the larger quantities of acid. Now one of these solutions, although quite acid in reaction, was unaffected by boiling. Mere neutralization therefore even with the aid of heat will not precipitate the proteid. I then made the following additional experiments. Into four test-tubes I dropped respectively 1, 2, 3 and 4 drops of calcium nitrate solution; I then poured 10 c.c. of the alkali albumin solution into each tube, and allowed the solutions to stand 20 hours. No change occurred in the solutions. They were all still alkaline in reaction.

On slowly raising the temperature of the water, the solution with 4 drops became particulate at 50° C.; the solution with 3 drops at 59° C.; the solution with 2 drops at 75° C.; but the solution with 1 drop was unaffected at boiling point. Nearly the whole of the proteid was precipitated in the three tubes, as I proved by duplicate experiments. Now in this last experiment, the solution with calcium chloride became particulate, although it was alkaline in reaction; and we have seen that with hydrochloric acid it was necessary to make the fluid acid before precipitation occurred, and that solutions decidedly acid will not precipitate at all even on boiling. But a more striking difference remains to be mentioned: I added ·5 °/₀ solution of caustic soda to all the solutions. To the solutions containing the largest quantity of hydrochloric acid, and that containing the largest quantity of calcium nitrate, I added 6 drops; to the other solutions 4 drops.

Both the tubes containing the higher quantities of hydrochloric acid and calcium nitrate became alkaline; the hydrochloric acid solution very faintly alkaline; the calcium nitrate solution strongly alkaline. The other two solutions precipitated by hydrochloric acid remained slightly acid; whilst the corresponding solutions with calcium nitrate were strongly alkaline. In a short time all the precipitates caused by the addition of hydrochloric acid and heat dissolved completely, whilst the solutions precipitated with the calcium nitrate remained unaffected. They were allowed to stand 24 hours and were just the same at the end of that time.

I then filtered all the solutions. The hydrochloric acid solutions filtered rapidly and left nothing in the filter paper, whilst the calcium nitrate left a copious deposit on the filter. I then saturated the filtered solutions with ammonium sulphate, and obtained an abundant deposit with the hydrochloric acid solutions, and very little indeed with the calcium nitrate solution.

I made another series of experiments, using 10 c.c. of the albumin solution and 3 drops of the calcium nitrate solution in each of the 4 experiments, then heated and produced an abundant deposit. I then added to the four tubes respectively 2, 4, 8 and 16 drops of ·5 °/₀ caustic soda solution and allowed the tubes to stand 24 hours. The deposited proteid was only affected in the tube containing 16 drops of the alkaline solution. The fluid became less particulate and viscid; but still on filtering I found that only about the same amount of proteid passed through as with smaller quantities, and about the same that passes through from a solution of alkali albumin precipitated by 3 and 6 drops of calcium chloride solution, to which no caustic soda has been added.

In another series of experiments 5 test-tubes received 10 c.c. of this alkali albumin mixture, and into these tubes respectively I dropped 1, 2, 3, 4 and 5 drops of ·5 °/₀ solution of caustic soda, and into each tube 4 drops of calcium nitrate solution. On boiling, all the solutions became particulate and then solid. After the experiment the solutions were strongly alkaline.

I record another series of experiments.

After diluting 50 c.c. of white of egg to 400 c.c. with ·1 °/₀ caustic soda I slowly heated and boiled the mixture. I next added some 1 °/₀ solution of hydrochloric acid, leaving the mixture strongly alkaline, and found that it required 12 drops of 1 °/₀ hydrochloric acid to 10 c.c. of the mixture to make it just faintly acid.

I then made a series of experiments using 10 c.c. of the mixture in each. Into the first three test-tubes I dropped respectively 3, 4 and 6 drops of 10 °/₀ solution of calcium nitrate: the solutions remained strongly alkaline. To the remaining three test-tubes I respectively added gradually 12, 14 and 16 drops of 1 °/₀ solution of hydrochloric acid: each of the solutions gave an acid reaction, the first being faintly acid. The first of these solutions remained unchanged; the second became opalescent and the third a little milky, but not particulate: after standing 24 hours no further change ensued. The tubes were then placed in a bath and the temperature slowly raised. The solution containing 6 drops of calcium nitrate solution became opalescent at 50° C., particulate at 72° C., and set into a jelly at 76° C. The solution with 4 drops underwent similar changes at 63° C., 88° C. and 92° C. The solution with only 3 drops became opalescent at 79 and a little milky at boiling, but not particulate. The solution with the largest quantity of acid became particulate at 88, and remained slightly particulate after boiling. The solution with 14 drops of dilute hydrochloric acid became a little milky, but not particulate; the solution with 12 drops after boiling was a little opalescent.

In these experiments the calcium salt precipitated the proteid from a decidedly alkaline solution. No doubt a double decomposition took place, $Ca\,(HO)_2$ being formed. But calcium hydrate readily dissolves alkali albumin. For if alkali albumin is precipitated and dissolved by excess of acid, on adding calcium hydrate solution, the alkali albumin is of course precipitated, and a very slight excess redissolves the precipitate.

Finally I made alkali albumin by diluting white of egg with 7 parts of ·1 % caustic soda and filtered and then dialysed into distilled water for four days. At this time the solution was neutral, but was not precipitated.

I added to 10 c.c. of the albumin mixture 2 drops of ·5 % solution of caustic soda, and then 4 drops of calcium nitrate solution. The solution had a decidedly alkaline reaction. On heating, a copious deposit occurred at 75° C.

Here again the calcium salt caused a deposit in an alkaline solution. As in the previous experiment, a double decomposition took place $Cu(HO)_2$ being formed : but, as I have just shown, lime water readily dissolves alkali albumin.

Summary.

I. Calcium salts do not promote heat coagulation of albumin.

II. They cause heat coagulation of alkali albumin.

III. In some solutions they act partly by lessening the alkalinity of the solution.

IV. They have however a further and more powerful action, for they precipitate alkali albumin in strongly alkaline solutions. Whether they act by diminishing the solvent power of the menstruum, or whether they alter the proteid molecule and so render it less soluble, these experiments do not explain.

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THE INFLUENCE OF CARBONIC ACID DISSOLVED IN SALINE SOLUTIONS ON THE VENTRICLE OF THE FROG'S HEART. BY SYDNEY RINGER, M.D., F.R.S.

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IN this communication[1] I desire to draw attention to the influence of carbonic acid dissolved in distilled water on the frog's heart, when perfused as saline solution.

Distilled water exposed to the air freely absorbs carbonic acid, giving it a distinct acid reaction. This carbonic acid greatly lessens the time that saline solution can sustain the ventricle's contractility.

To remove the carbonic acid the water was boiled about three hours; then cooled and made into ·6% saline solution and at once or on the next day[2] used in the experiment.

Of course oxygen is driven off by heat as well as carbonic acid but I shall show that oxygen dissolved in saline apparently exercises no influence in sustaining or impairing cardiac contractility.

Distilled water always gives a distinct acid reaction with delicate litmus paper, and after boiling for three hours the water though less acid still generally remains slightly acid, and on only two occasions have I obtained a neutral water.

Whilst with ordinary saline (i.e. made with distilled water which has been exposed to the air and has absorbed carbonic acid) the contractility of the heart quickly grows weak and is soon suspended; with saline made with water recently boiled, contractility persists much longer. The length of time however varies within rather wide limits. If the circulation is rapid the beats at first grow weak but in a short time grow stronger again and may almost regain their original completeness. Then the contractions again grow weak and in a variable time, from 20 to 50 minutes, contractility ceases, even after strong induction shocks. The average duration of 14 experiments was 28 minutes.

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[1] In these experiments I employed Roy's tonometer.

The various solutions of calcium, sodium and potassium were of 1% strength. Most of these experiments were made in June, July and September.

[2] In some experiments the flask containing the boiled distilled water was closed with a cork through which a glass tube was passed and this was connected with a bottle containing a concentrated solution of caustic potash, so that as the fluid cooled the air drawn into the flask was freed from carbonic acid.

In hot weather the heart beats rapidly, and some fusion and tonic contraction occurs which becomes less as the rhythmic contractions grow weaker. In some instances the saline prolonged both contraction and dilatation, especially dilatation, and occasionally when the contractions became very weak, they were also irregular.

The influence of carbonic acid is well shown by passing washed carbonic acid through distilled water that has been boiled. This without carbonic acid as saline permits contractility for half-an-hour or longer, whilst some of the same saline containing a large quantity of carbonic acid arrests the ventricular contractions very soon after the solution reaches the heart. Perfusing the arrested ventricle with saline solution free from carbonic acid does not restore contractility. Neither does the addition of various neutral salts (calcium, potassium or sodium) to the saline containing carbonic acid restore contractility. Still contractility is not destroyed, it is only suspended, for if a circulating saline made of tap-water (New River Water Company) and to 100 c.c. of which 4 c.c. of 1 °/$_0$ solution of potassium chloride is added contractility on electrical stimulation and sometimes spontaneously, returns and becomes after a time complete. The tap-water circulating fluid contains a considerable quantity of lime as sulphate and bicarbonate and has an alkaline solution. This fluid no doubt neutralizes the carbonic acid, frees the ventricle from its influences when the calcium and potassium salts are sufficient to restore contractility.

I record one experiment:

Saline charged with carbonic acid rapidly arrested contractility, although the ventricle was powerfully stimulated by induction shocks. The addition to 100 c.c. of 4 c.c. calcium nitrate solution and next 2 c.c. of potassium chloride solution, and lastly of 2 c.c. of sodium bicarbonate solution failed to restore contractility. The solution gave an acid reaction. After adding ·5 °/$_0$ solution of sodium hydrate till the fluid became faintly alkaline (and this was added so slowly that an hour elapsed) contractility returned and slowly improved. In this experiment the carbonic acid quite prevented the calcium, sodium and potassium salts restoring contractility, but on neutralizing the carbonic acid contractility returned.

The effect of carbonic acid again is shown by its influence on various solutions. Tap-water circulating fluid and saline made with boiled distilled water 100 c.c. containing 4 c.c. calcium nitrate, 2 c.c. potassium chloride and 2 c.c. sodium bicarbonate solutions are both excellent circulating fluids, sustaining the ventricle for more than two hours with scarcely any impairment in the strength of the beats. Yet if carbonic acid is passed through either of these solutions as they perfuse, it very speedily suspends contractility. These fluids when treated with carbonic acid give a double reaction; delicate litmus paper is first reddened, but on exposure gives a distinct alkaline reaction from the escape of carbonic acid. After suspending contractility on returning to tap-water circulating fluid perfect contractions return.

Saline solution containing much carbonic acid, to which a large quantity of sodium bicarbonate solution is added so that the solution to delicate litmus paper gives first an acid reaction and then on exposure a strong alkaline reaction, rapidly suspends contractility.

A circulating fluid containing one part serum (reddened with hæmoglobin) to two parts saline, through which washed carbonic acid was passed quickly suspended contractility. The carbonic acid must be passed through such a solution for a considerable time to arrest contractility and then the fluid gives the double reaction with litmus, first reddening it and on exposure of the paper turning it blue.

I next adopted the obvious plan of getting rid of the acidity by neutralization with weak solution of caustic soda or caustic potash. Some of the saline solutions were made nearly as possible neutral, others remained faintly acid. These solutions gave unexpected results. The contractility was long maintained but the character of the contraction was greatly changed. In about 20 minutes the contractions became weaker and their duration much prolonged both in contraction and dilatation. At the same time there occurred tonic contraction. The contractions then gradually grew weaker, tonic contraction persisting, but relaxing a little as the rhythmic contractions became weaker. These effects also occur in the addition of small quantities of sodium bicarbonate to saline made with boiled distilled water.

When the ventricle perfused with saline containing either sodium hydrate or potassium hydrate has almost ceased contracting, the addition of potassium chloride removes tonic contraction and arrests the feeble beats, but the addition of calcium nitrate restores good contractions.

The oxygen driven off on boiling distilled water apparently has no influence on the ventricular contractions, for saline solution through which oxygen was passed for a considerable time did not in any way affect the contraction. It behaved just as saline made from recently boiled distilled water.

In a previous paper I have shown that a circulating fluid capable of long sustaining the ventricular contractility is made by allowing saline solution to stand over tribasic phosphate of calcium[1], to each 100 c.c. of the saline adding 2 c.c. of potassium chloride or acid potassium phosphate (HK^2PO^4) solution. No doubt the phosphate of calcium effects its influence on the ventricular contractility in two ways. It precipitates most of the carbonic acid of the distilled water by giving up a portion of its calcium: precipitating carbonate of lime. Whilst the small quantity of acid phosphate of calcium that becomes dissolved is sufficient with the aid of the potassium salt to sustain the ventricular contractility for a considerable time.

I shall next treat of the influence of various salts added to saline made of boiled distilled water.

When the ventricle has grown very weak or contractility is lost the addition of 2 c.c. of sodium bicarbonate solution to 100 c.c. slowly restores contractions: and induces tonic contraction. On adding ·4 c.c. to ·5 c.c. potassium chloride solution, the tonic contraction ceases and the excursion of the trace becomes greater, but this is mainly due to increased dilatation of the ventricle from the relaxed tonic contraction, but the trace too rises a little higher than previous to the addition of the potassium chloride, but this depends on the potassium chloride slowing the spontaneous beats and giving longer time for reparation, for if the contractions are excited more frequently then the trace is no higher than before the addition of potassium chloride. In a short time the contractions grow weak and contractility ceases. When potassium has slowed the ventricle and increased the excursion of the trace dilatation is greatly delayed.

The addition of ·5 c.c. of potassium chloride solution to 100 c.c. saline after the ventricular beats have become weak soon arrests contractility

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[1] I find the saline with calcium phosphate is rather more efficacious if the fluid is used (with potassium chloride) before the powder has completely settled and whilst the fluid is still slightly milky.

When the ventricle perfused by saline made with boiled distilled water ceases to contract the addition of a calcium salt (2 c.c. to 4 c.c. calcium nitrate solution to 100 c.c. saline) speedily restores contractility, but the contraction is greatly prolonged, especially the dilatation, and much tonic contraction is induced, so that the ventricle is soon thrown to persistent contraction. The addition of potassium chloride solution (1·5 c.c. to each 2 c.c. of calcium nitrate solution) gradually removes the tonic contraction and induces complete contractions, and on the addition of 2 c.c. sodium bicarbonate solution the ventricle will continue to contract perfectly for more than two hours, this being the longest time that I have continued the experiment.

Conclusions.

Free carbonic acid dissolved in saline arrests cardiac contractility. Whether it has a specific effect or acts as an acid these experiments do not decide.

Saline made of boiled distilled water, from which therefore most of the carbonic acid is expelled, permits the ventricle to contract longer than saline made of unboiled distilled water.

Saline made of boiled distilled water however suspends contractility on an average in twenty to thirty minutes and often in twelve to fifteen minutes.

Fluids well suited to sustain cardiac contractility for a long time, if treated by carbonic acid passed through them are no longer able to sustain contractility. This applies even to a mixture of blood serum and saline (1 to 2).

Experiments with saline made of boiled distilled water corroborate my previous experiments.

Potassium chloride added to saline is inadequate to sustain contractility.

Sodium bicarbonate induces tonic contraction whilst the contractions at the same time grow weak.

The combined addition of sodium bicarbonate and potassium chloride cannot sustain contractility. Calcium salts as calcium nitrate added to saline restores contractions but dilatation becomes greatly delayed and some tonic contraction is induced. The delay in dilatation and tonic contraction are obviated to a suitable quantity of potassium chloride.

A combination of calcium nitrate and potassium chloride will long sustain the cardiac contractility, but the solution is made still more efficient by adding sodium bicarbonate (saline 100 c.c., calcium nitrate 4 c.c., potassium chloride 2 to 3 c.c., sodium bicarbonate 2 c.c.).

Calcium and potassium salts are mutually antagonistic.

If potassium chloride is in slight excess then after a suspension of contractions, on renewing the contractions a staircase of beats occurs which is quite removed on adding more calcium salt.

If calcium salt is slightly in excess, the contraction is prolonged and dilatation delayed, and these effects are most marked after a long suspension of contractions and may disappear on contractions being frequently induced.

THE ACTION OF POTASSIUM, SODIUM AND CALCIUM SALTS ON TUBIFEX RIVULORUM.

By SYDNEY RINGER, M.D., F.R.S., AND HARRINGTON SAINSBURY, M.D., F.R.C.P.

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PREVIOUS experiments, for the most part recorded in the volumes of this Journal, have established the important part played by lime in the functions of living tissues. As an instance, we may mention the presence of lime as a factor essential to the contraction of cardiac muscle. The experiments demonstrating this shewed also the antagonism which existed between the influence of lime and of potassium salts. This was tested both physiologically[1] and toxically[2]. In a long series of experiments lime salts were shewn to be necessary to the act of clotting of blood and to the curdling (clotting) of milk, and again an active antagonism was found to obtain between the salts of lime and of potassium. True the clotting of blood can scarcely be classed as a vital process, still less the clotting of milk, yet it is difficult to dissociate vital from non-vital processes and a characteristic potency or function of a tissue may probably depend as much on a katabolic (disintegrating) as upon an anabolic (integrating) movement, we may therefore allow these experiments to stand here.

The influence of lime in purely non-vital processes, a physico-chemical action therefore, has also been tested in one direction, its power, viz., to affect the swelling of dried Laminaria when placed in watery solutions. Lime was found to control markedly the powers of imbibition, the Laminaria swelling out to a much greater degree when placed in distilled water than when a small percentage of a lime salt had been added. These experiments may have some bearing upon those about to be described.

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[1] This *Journal*, Vol. IV. p. 29, 1883.

[2] This *Journal*, Vol. IV. p. 247, 1883.

The present set of experiments were undertaken in order to test the influence of salts of lime and potash and incidentally of soda also upon *whole* living organisms. The presence or absence of motor power was the chief test applied, but the organisms were also watched as to the presence of disintegration and its degree. The Tubifex Rivulorum (Lamarck) was chosen as suitable for the purpose. It is an actively moving minute worm tending, with its fellows, to form dense clusters or balls of filaments, each thread being in continuous wriggling movement. The structure, so far as it concerns us may be very briefly given[1]:—The integument, ringed and beset at intervals with minute bristles or hairs, consists of a cuticle, homogeneous in structure, and intimately united with an underlying corium. Internally this is bounded by a subcutaneous layer of muscular fibres in two layers, circular and longitudinal, of which the latter is the more developed. The contractions of the subdermal fibres cause the movements of the body. Traversing the body from end to end is the digestive tube and in close connection is the vascular system consisting of two longitudinal main trunks, dorsal and ventral, united by lateral branchings, a pair for each ring. Two so-called hearts are found near the head end on either side of the dorsal vessel and uniting it with the ventral vessel. In life the contractions of these and of the dorsal vessel are plainly visible under a low power through the transparent body wall. A perivisceral space intervenes between the body wall and the above tubes and transverse partitions one for each ring divide this space into compartments. Other structures, reproductive, secretory, we may omit. The digestive tube shews a very delicate mucous lining the surface of which is covered by a ciliated epithelium. There is also a delicate muscular coat surrounding the mucosa to which the peristaltic movements of the tube are due.

In tap water, renewed from time to time, the Tubifex will live for an indefinite period, exhibiting all the signs of an active vitality.

The following is the method of experimentation adopted: a series of beakers, each containing the same volume of fluid, received each a number of the worms, these were then watched and a record kept of their condition, in particular as to movement and disintegration. By varying the proportions of the salines added to the beakers it was possible to obtain an indefinite variety of separate series. In this way the effects of the salts, potassium and sodium chloride, calcium nitrate and phosphate, sodium oxalate were tested. Distilled water and tap water were the fluids used, and to which these salts were added—the tap water is that supplied by the New River Company.

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[1] *Histoire naturelle du Tubifex des ruisseaux*, par Jules d'Udekem. Bruxelles, 1853; *Structure of Tubifex.* McIntosh, 1871.

We may now proceed to the actual experiments and first as to the effects of these two waters. In tap water, as has been already mentioned, the Tubifex will live for an indefinite period in full activity, this will be seen again and again in the experiments to be recorded and to which the tap water beaker served as the control. It will be noted however that the creatures, even in this medium, may not escape some degree of disintegration. This when present affects only a small percentage of the organisms and does not appear till late in the experiment, i.e. after some days, and it probably selects those only which have suffered mechanically in the act of transference, or in the act of separating the clustered colony. The separation was effected by allowing the tap to play upon them with moderate force, the transference to the test-medium by means of a pipette.

Distilled water acts very differently. In this medium the organisms continue for a time their active movements but gradually disintegration appears and ultimately they are all converted into a granular débris. The movements may continue for some hours becoming gradually more sluggish and the disintegration slowly advancing—within 24 hours the latter process is generally complete. The action of distilled water will be illustrated again and again in the experiments which follow.

If now we modify distilled water by the addition of salines we modify greatly the action of the medium. We will first consider potassium chloride.

On Sep. 29th, 1893 the following experiment was made:—five beakers were taken and received the following quantities of fluid:

I. 200 c.c. of distilled water
II. ,, ,, + 0·5 c.c. of a 10 $^0/_0$ sol. of potassium chloride
III. ,, ,, + 1 c.c. ,, ,, ,,
IV. ,, ,, + 1·5 c.c. ,, ,, ,,
V. ,, ,, + 2 c.c. ,, ,, ,,

Into each by means of a pipette several of the Tubifex organisms were introduced.

The experiment began at 2.15 P.M.

In 19 hours though disintegration was well marked in I., some of the organisms were active and even after 43 hrs. there were still some which were active though most were disintegrated. The action of distilled water was very exceptionally slow here.

In the beakers III., IV., V., the organisms were motionless within 19 hrs. (one Tubifex only was moving in beaker IV.).

In beaker II. after 43 hrs. all were motionless except one. A few only were disintegrated in this beaker.

When rendered motionless recovery experiments were made but these we omit for the present.

On October 3rd the following series was arranged:

I. 200 c.c. of distilled water + 2 c.c. of a 10 $^{0}/_{0}$ solution of KCl
II. ,, ,, + 2·5 c.c. ,, ,, ,,
III. ,, ,, + 3 c.c. ,, ,, ,,
IV. ,, ,, + 3·5 c.c. ,, ,, ,,
V. ,, ,, + 4 c.c. ,, ,, ,,

The experiment was started at 9 A.M.

At 2 P.M., i.e. after 5 hours, a few organisms were moving slightly in beakers I., II., III. There was no movement in IV. and V.

At 5 P.M., i.e. after 8 hours, there was no movement in I., II., III.

All the motionless organisms were subsequently recovered.

On September 22nd a series was arranged to test the action of KCl and NaCl, separately, in modifying the action of distilled water.

I. 200 c.c. distilled water
II. ,, ,, + 1 c.c. KCl solution, 10 $^{0}/_{0}$
III. ,, ,, + 2 c.c. ,, ,,
IV. ,, ,, + 1 c.c. NaCl ,, 10 $^{0}/_{0}$
V. ,, ,, + 2 c.c. ,, ,,
VI. ,, ,, + 1 c.c. KCl sol., 10 $^{0}/_{0}$ + 1 c.c. NaCl sol. 10 $^{0}/_{0}$.

I. After 24 hours most of the organisms were disintegrated, but there was still one moving after 48 hrs.

II. In 4 hrs. movements only just perceptible, in 8 hrs. motionless. No disintegration after 48 hrs.

III. In 4 hrs. slight movement only; in 8 hrs. one moving slightly; next morning, i.e. after 24 hrs., all motionless. No disintegration after 48 hrs.

IV. After 8 hrs. still active; after 24 hrs. motionless, some disintegration; after 48 hrs. all disintegrated or disintegrating.

V. After 8 hrs. active; after 24 hrs. very sluggish, but not disintegrated, after 28 hrs. same state; after 48 hrs. motionless but not disintegrated.

VI. After 4 hrs. sluggish; after 8 hrs. motionless; after 24 hrs. very slight movement was detected—no disintegration; after 48 hrs. no disintegration.

These experiments shew first the disintegrating power of distilled water and next the paralyzing power of potassium chloride with suspension or retardation of disintegration. Sodium chloride in the smaller dose of ·05 p.c. retards but does not suspend disintegration, it contrasts with potassium chloride in its relatively slight action upon movement—in the larger dose (·1 p.c.) disintegration is more effectually checked, but the effect on movement is even less marked, still the solution does slowly paralyze. In the effect of potassium and sodium chloride combined—the potassium action predominates in respect of paralysis, the fluid behaving quite like the solution containing 1 c.c. of KCl alone: disintegration, as we should expect is held in check.

On September 24th a longer series of KCl and NaCl solutions in distilled water gave results confirmatory of those just recorded. These results were as follows:—

Disintegration, complete, by the distilled water within 24 hrs.

In the KCl beakers nearly complete paralysis in $4\frac{1}{2}$ hours, only one or two were moving sluggishly in each beaker; within 24 hrs. all were motionless. The beaker containing 1 c.c. each of KCl and of NaCl combined acted just like the 1 and 2 c.c. of KCl alone. In the NaCl beakers 1, 2 and 3 c.c. of NaCl 10 $^0/_0$ were contained. The influence upon movement was very slight, thus after 10 days there was some movement in two of the flasks containing respectively 1 c.c. and 2 c.c. In two other flasks containing these same quantities of NaCl movement was abolished in six days, for this discrepancy individual differences in vitality of the organisms might easily account, but either result, 6 or 10 days, contrasts sufficiently with the paralyzing action of potash made manifest in a few hours. The flask containing 3 c.c. NaCl gave practically the same results as those containing 1 and 2 c.c., so far as movement was concerned. As to disintegration this was held in check by the NaCl, thus after 6 days all the organisms in the NaCl flasks retained their shape. Under the microscope however the organisms in one flask, containing 2 c.c. NaCl, and which had lost their colour, shewed an absence of structure; in them some internal disintegration had evidently occurred. In the flask containing 3 c.c. NaCl preservation at the same stage was more complete, the organisms retained their red colour and though motionless to the naked eye the microscope shewed slow movements of the whole body and also contractions of the vascular canal.

We pass now to the influence of lime salts. We have seen that tap water will support life for an indefinite period. This fitness must be held to depend upon the presence of lime as one essential factor. Thi appeared in many experiments and first in the power of distilled water + nitrate of lime, and distilled water + phosphate of lime to support vitality. These two solutions seemed to replace tap water fairly satisfactorily. One or two cubic centimetres of a 10 % solution of nitrate of lime added to 200 c.c. of distilled water will be ample for the purpose, or a saturated solution of phosphate of lime in distilled water. The latter salt is so very insoluble that this saturated solution really contains less lime than is present in tap water, indeed the usual statement is that calcium phosphate is insoluble. We speak of the tricalcium salt. From the fact that tricalcic phosphate can act in such minute quantity, we must conclude that the amount of calcium nitrate employed by us was very excessive, in fact may be regarded as a luxus supply. This point we have not sought to determine quantitatively. Some of the experiments with a lime salt in distilled water seemed to suggest that the Tubifex suffered more disintegration than was observed with tap water, but when this process is slow, as it was in the lime experiments, it is difficult to make sure that the process is not rather due to some damage done to the organisms or to a weak vitality of the same. Even in tap water, in the course of an experiment extending over several days, disintegration was observed, it was generally slight but sometimes more marked.

The first proposition then, respecting lime salts, is that they will convert distilled water into a medium capable of supporting the vitality of the Tubifex for considerable periods, e.g. several days.

Next, it was found that lime, in the same proportions, was able to inhibit or antagonize the paralyzing action of potassium chloride. This was made quite definite by a number of experiments in which calcium nitrate or calcium phosphate was introduced along with potassium chloride. Tap water acted in the same way as a solution of a lime salt in distilled water and the inference was that it is the lime present in tap water which acts thus.

Thirdly lime was able to remove the effects of potash already produced, e.g. having paralyzed the organisms by potassium chloride it was ssible to recover them quickly by the addition of a calcium salt.

rganisms which have been paralyzed by potassium chloride may be to recover quickly by transferring them to a solution of a lime in distilled water or to tap water simply, but the recovery in these s might be due solely to a diffusion out from the tissues of the alyzing salt, KCl, and not to a direct antagonizing lime action, and is probable that both these factors do come into play. To prove wever that lime can antagonize without the aid of any diffusion the llowing experiment was made.

Oct. 1st. Four beakers were charged.

I. 200 c.c. distilled water + 1 c.c. of a 10 % solution of KCl
II. ,, ,, ,, ,, ,,
III. ,, ,, + 2 c.c. ,, ,,
IV. ,, ,, ,, ,, ,,

Thus II. and IV. were exact replicas of I. and III.

The organisms now introduced were found motionless in each case at the end of 7 hrs.

I. now received 1 c.c. of a 10 % solution of $Ca(NO_3)_2 = I^a$.
II. the organisms were transferred to a flask containing tap water to which KCl had been added so as to make it of the same KCl strength as the original solution = II^a.
III. received 2 c.c. of a 10 % solution of $Ca(NO_3)_2 = III^a$.
IV. remained unchanged.

After 17 hours from the transferences the organisms in I^a, II^a, and III^a, were all found active. In flask IV. the organisms were quite motionless.

Now to flask IV. 2 c.c. of $Ca(NO_3)_2$ solution 10 % were added, and within seven and a half hours from this there was some recovery and within 23 hours of the transference many organisms were active. This recovery was from a motionless state of 18 hours' duration.

Repeating these experiments on Oct. 2nd, completely confirmatory results were obtained, and it is thus clear that the lime directly and effectually antagonizes the potassium salt.

In an experiment made on Oct. 3rd, larger quantities of KCl were used, viz. 2 c.c., 2·5 c.c., 3 c.c., 3·5 c.c. and 4 c.c. of a 10 % solution. The organisms having been rendered motionless corresponding quantities of $Ca(NO_3)_2$ solution, 10 % were added and there was noted in each case recovery within a few minutes. The massive dose of the potassium salt was rapidly overcome by the massive dose of the lime salt. Recovery also took place in tap water which contained the same proportions of KCl as the above, but the recovery was much slower; the addition to the tap water of a massive dose of lime salt accelerated the recovery.

The lime effect was tested in yet another way by the addition viz. of sodium oxalate to distilled water. Of the oxalate varying quantities were used, viz. 0·5 c.c., 1 c.c., and 1·5 c.c. of 10 % solution—and three other flasks received the corresponding quantities of sodium oxalate + in each case 2 c.c. of 10 % solution of NaCl.—Each flask contained 200 c.c. of distilled water.

The organisms in the flasks containing 1·5 c.c. of sodium oxalate solution became almost motionless in 8½ hours, but after 24 hours there was still some slight movement present, however on transferring the organisms to tap water the movements greatly increased in a few minutes and thence onward they remained active. The 0·5 c.c. sodium oxalate flasks did not seem to influence the organisms, the 1 c.c. flasks caused sluggishness of movements in one case but not in the other.

The influence of sodium oxalate here is probably by withdrawing lime from the tissues or by rendering it useless within the tissues by precipitation.

These experiments were not made exhaustive.

Sodium oxalate seemed to completely prevent disintegration, during a period of three days (78 hrs. strictly).

To sum up, these experiments shew clearly :

1st. That a lime salt is essential to the vitality of the organism, Tubifex Rivulorum.

2nd. That whilst a very minute quantity of a lime salt seems to be adequate to the maintenance of this vitality, the quantity may be increased enormously without producing any very definite result—in other words, it is very indifferent to the tissues. Contrast here the quantity of lime present in a solution of tricalcic phosphate with that contained in 2 or 3 c.c. of a 10 °/₀ solution of calcium nitrate.

The minute dose of lime will represent the physiological one; the massive dose, the therapeutic or toxic one, only we did not reach the toxic limit.

3rd. That lime both in the minimal and in the massive dose antagonizes the paralyzing influence of a potassium salt, and that whilst a minimal dose of lime seems to have extraordinary powers of inhibiting or antagonizing the action of large quantities of potash, a massive dose of the potassium salt seems to be easiest overcome by a massive dose of the calcium salt.

4th. That sodium contrasts with potassium in the relative feebleness of its action—maintaining its character as an indifferent element.

We may add that all the salts potassic, sodic, calcic used in these experiments hold in check the disintegrating influence of distilled water. This action is probably physico-chemical. The experiments on this point shew :—

5th. The remarkable power which a minute dose of a lime salt, e.g. of lime phosphate, possesses in maintaining the integrity of the tissues. We may here again refer to the experiments already quoted in which it was found that the powers of imbibition of Laminaria were notably controlled by a lime salt. The quantities of sodium and potassium salts which also controlled the disintegrating process were maximal as compared with this quantity of the lime salts.

In what light we are to regard the physiologic and toxic antagonism existing between lime and potash it is hard to determine. That lime does not act by preventing the ingress of the potassium poison by imbibition or otherwise is clear from those experiments in which the potassic effect was first produced and then overcome *in situ* as it were, by the calcium salt. If we say that the molecular vibrations of the calcium salts do not harmonize with those of the potassium salts, and by mechanical interference annul each other's action in the same way that waves of light of different rates of vibration interfere with each other and may produce a negation of effect—viz. darkness—if we say this, we say nothing that really advances the understanding of the subject.

THE INFLUENCE OF SALINE MEDIA ON THE TADPOLE. BY SYDNEY RINGER, M.D., F.R.S., AND ARTHUR G. PHEAR, M.B. *Cantab.*

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FOR some years we have been studying the action of various salts on living tissues, and we have found them to possess at least two distinct properties. Some salts are efficient in maintaining structural integrity of tissue; others are able to support functional activity. The two properties are not necessarily combined in a single salt: a salt which is competent to support function may be very inefficient or quite incapable of maintaining integrity.

We have shown[1] that the gill edges of water mussel if placed in distilled water quickly disintegrate, the cells becoming swollen and granular, and separating from one another. Many of the cells burst, and ciliary action ceases. The addition of a small quantity of calcium chloride or of sodium bicarbonate is sufficient to sustain both integrity and function for many days.

We have shown too that distilled water causes separation of the cuticle from fishes and newts[2].

In the present communication we shall put forward the results of further observations on the action of various salts on the intact organism. The organism employed has been the tadpole, those only being selected in which development had progressed beyond the gill stage.

The following is the method of experiment adopted. In each one of a series of beakers 200 c.c. of distilled water were placed. To the first of these nothing further was added. To the other beakers were added increasing quantities of a solution of the salt under examination, so that a series was obtained of solutions of ascending strength. Into each of these vessels three or more tadpoles were introduced, and the action of the solutions on the tadpoles was observed and duly noted.

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[1] Ringer and Buxton. "Concerning the Action of small quantities of Calcium, Sodium, and Potassium salts upon the Vitality and Function of Contractile Tissues and the Cuticular Cells of Fishes." This *Journal*, VI. 154. 1885.

[2] Ringer. "The Influence of Saline Media on Fish, etc." This *Journal*, IV. VI. and V. 98.

Tadpoles placed in distilled water rapidly become motionless, after a period varying as a rule between two and six hours. Paralysis is quickly followed by disintegration; indeed signs of desquamation may be observed before movement has entirely ceased; and we believe it to be by means of such disintegration that distilled water causes death. Certain salts when added to distilled water in proper proportion sustain motion in tadpoles, in some cases for many days. This fact of itself is no matter for surprise; since were it not for the sustaining properties of some salts tadpoles could not exist in the natural waters of rivers and ponds. Some of the salts which we have found to sustain are however powerful poisons, such as hydrocyanic acid, and cyanide of potassium, and the neutral oxalate of potassium. As illustrating this property of supporting life, experiments with potassium ferrocyanide and potassium oxalate will be described.

Exp. 1. *Potassium ferrocyanide.* In each beaker 200 c.c. of distilled water, and to all but beaker (1) potassium ferrocyanide added so as to make percentage of it in

(2) ·000125 p.c.
(3) ·00025 ,,
(4) ·0005 ,,
(5) ·00075 p.c.
(6) ·001 ,,

April 23. 10 a.m. Tadpoles introduced.
4 p.m. (1) All tadpoles motionless.
(2) One moves languidly. Two motionless.
(3)—(6) Quite active.
7 p.m. (2) All motionless.
(3)—(6) Quite active.
April 28. (6th day.) 10 a.m. All tadpoles in beakers (3)—(6) quite active, with exception of one dead in beaker (6).
Experiment discontinued.

Exp. 2. *Potassium ferrocyanide.* 200 c.c. distilled water in each beaker; so much of the salt added as to make percentage in

(1) ·00125 p.c.
(2) ·0025 ,,
(3) ·005 ,,
(4) ·0075 p.c.
(5) ·01 ,,

April 22. 11 a.m. Tadpoles introduced.
25. 10 a.m. Beakers (1)—(4). All active.
Beaker (5). One tadpole dead. Three active.

April 27. 10 a.m. (1)—(4) All active.
(5) All dead.
28. 10 a.m. (1)—(4) All active.

Experiment discontinued. Tadpoles in the first four beakers being as active after lapse of six days as at beginning of Exp.

Exp. 3. *Potassium oxalate* (neutral). In each beaker 200 c.c. distilled water; potassium oxalate added so as to make percentage of it in

(1) ·05 p.c. (3) ·1 p.c.
(2) ·075 „ (4) ·15 „

April 8. 10 a.m. Tadpoles introduced.
9. 10 a.m. (1)—(3) All active.
(4) Two motionless. One quite active.
12. 10 a.m. (1)—(3) Active.
(4) One still moving, but sluggish.

Experiment discontinued, tadpoles being alive and active after lapse of four days.

Many other salts have been found to possess similar properties, particularly salts combined with weak acids, such as the carbonates, citrates, tartrates, and oxalates. The results of numerous experiments with these may be tabulated as follows:—

Table A. *Salts which sustain for an indefinite period.*

Salt	Strength found to sustain	Duration of experiment	Duration of life in control beaker containing distilled water only
Potassium Bicarbonate	1 in 1000 to 1 in 500	8 days	Under 7 hours
„ Citrate ($KH_2C_6H_5O_7$)	1 in 20,000 to 1 in 1000	3 „	
„ „ ($K_3C_6H_5O_7$)	1 in 8000 to 1 in 700	3 „	
„ Tartrate ($KHC_4H_4O_6$)	1 in 10,000 to 1 in 1000†	6 „	
„ „ ($K_2C_4H_4O_6$)	1 in 1000†	4 „	
„ Oxalate ($K_2C_2O_4$)	1 in 4000* to 1 in 800†	5 „	
„ Phosphate (K_2HPO_4)	1 in 2000 to 1 in 150†	5 „	2½ hours
„ „ (K_3PO_4)	1 in 4000	5 „	2½ „
„ Cyanide	1 in 80,000	9 „	Under 3 hours
„ Ferrocyanide	1 in 400,000 to 1 in 15,000	6 „	
Sodium Chloride	1 in 300 to 1 in 200	7 „	
„ Sulphate (Na_2SO_4)	1 in 800*	5 „	
„ Bicarbonate	1 in 1000	8 „	Under 7 hours
„ Oxalate	1 in 4000*†	5 „	
„ Phosphate (NaH_2PO_4)	1 in 800†	5 „	3½ hours
„ „ (Na_2HPO_4)	1 in 3000 to 1 in 800†	5 „	3½ „
Calcium Bicarbonate‡		6 „	
„ Phosphate§		6 „	
Iron Citrate	1 in 8000* to 1 in 1500	2 „	
Caffein Citrate	1 in 8000* to 1 in 2000	3 „	

In all these cases the tadpoles were as active, and with vitality apparently as unimpaired, at the close as at the commencement of the experiments.

* Solutions more dilute than these were not tested.
† Stronger solutions were not tested.
‡ Prepared by passing CO_2 through lime water.
§ Prepared by allowing distilled water to stand for 24 hours on tricalcic phosphate [$Ca_3(PO_4)_2$]. The solution thus obtained contains less lime than is present in tap water.

The limits of dose here given represent those ascertained by repeated experiment with each separate salt. In a number of experiments, although the main results are similar, yet there is some slight variation in the limits of the dose which sustains, doubtless owing to the varying susceptibilities of individual tadpoles. It will be noticed how varied is the quantity required of these several salts, and what a very minute dose of some is sufficient to support life. Potassium ferrocyanide is the most striking instance of this, a solution containing ·00025 p.c. being sufficient to maintain life and movement for a period exceeding six days. Since such minute quantities suffice, it might be supposed that the salts requisite for sustenance are derived from the fæces, which often are expelled in considerable quantity. Two considerations disprove this; first, the tadpoles do not survive in distilled water, although here as much fæcal matter is ejected as in the solutions which sustain; secondly, the tadpoles survive when transferred to a fresh solution, as soon as contamination with fæcal matter has occurred.

Other salts though not sustaining for these long periods, yet present a distinct superiority over distilled water in their powers of preserving life. These may be tabulated as follows:—

TABLE B. *Salts which sustain, but not indefinitely.*

Salt	Strength of solutions which support life	Duration of life in these solutions	Duration of life in distilled water
Sodium Sulphate (NaH_2SO_4)	1 in 7000	1 day	
,, Phosphate (Na_3PO_4)	1 in 4000 to 1 in 2000	3 days	3½ hours
,, Sulphite	1 in 700 to 1 in 400	3 ,,	
,, Arseniate	1 in 4000 to 1 in 900	1 day	Under 7½ hours
Ammonium Oxalate	1 in 4000 to 1 in 1000	4 days	

In some of these experiments it has happened that of four tadpoles some have become motionless within a short time, the remaining ones surviving for some days, even though transported into a fresh solution away from their dead companions.

In the case of a few salts we have been unable to discover any sustaining property, in spite of repeated trials and the employment of doses varying widely in range. Indeed, these salts seem to cause paralysis of movement and death sooner than distilled water. It will be noticed that many of these are potassium salts, and in association with the stronger acids.

TABLE C. *Salts which kill as soon as or sooner than distilled water.*

Salt	Solution in which tadpoles survive longest	Duration of life in these solutions	Duration of life in distilled water
Potassium Chloride	1 in 20,000	4 hours	4 hours
,, Sulphate ($KHSO_4$)	1 in 1500	3¾ ,,	
,, ,, (K_2SO_4)	1 in 40,000	3 ,,	
,, Nitrate	1 in 4000 to 1 in 1500	20 mins.	
,, Chlorate	1 in 20,000 to 1 in 400	1¼ hours	Over 1¼ hours
,, Acetate	1 in 4000 to 1 in 700	1½ ,,	
,, Oxalate (KHC_2O_4)	1 in 4000	5 ,,	
,, Phosphate (KH_2PO_4)	1 in 800	2 ,,	2½ hours
Sodium Acetate	1 in 400	5¼ hours	
Calcium Chloride	1 in 3500 to 1 in 1500	6 ,,	
,, Sulphate	Sat. sol.	3 ,,	

It was with some surprise that we found that acids, inorganic as well as organic, support life even for days when employed in appropriate doses. With stronger doses of the inorganic acids paralysis supervenes very quickly, desquamation commencing even before death, and progressing at a very rapid rate. The caustic alkalies also were found efficient to sustain in doses which are large as compared with the extremely minute quantities of acid which suffice. The results of our experiments with acids and alkalies are summarised in the following table:—

TABLE D. *Acids and Alkalies.*

Acids	Strength of solutions found to sustain	Duration of life in these solutions	Duration of life in distilled water
Hydrochloric Acid	1 in 70,000 to 1 in 50,000	4 days	2 hours
Sulphuric Acid	1 in 200,000 to 1 in 80,000	2 ,,	5 ,,
Nitric Acid	1 in 100,000 to 1 in 50,000	9 hours	2½ ,,
Acetic Acid	1 in 20,000	1 day	
Citric Acid	1 in 80,000 to 1 in 15,000	2 days	
Tartaric Acid	1 in 20,000	2 ,,	
Oxalic Acid*	1 in 20,000	3½ hours	
Sulphurous Acid	1 in 1000	2 days	Under 4 hours
Hydrocyanic Acid	1 in 500,000	3 ,,	
,,	1 in 1,000,000	1 day	
,,	1 in 4,000,000	9 hours	Under 6 hours
Alkalies			
Potassium Hydrate	1 in 4500 to 1 in 2000	1 to 2 days	4 hours
Sodium Hydrate	1 in 5000 to 1 in 3000	1 to 2 ,,	4 ,,

* No supporting action discovered here; but minute doses were not tested.

The foregoing experiments and tables show clearly that certain substances when added to distilled water change this from a medium in which tadpoles live only for a few hours to a medium capable of maintaining life for a much longer time. Whatever be the precise value of the doses indicated,—and we believe that the dose must vary within wide limits according to the stage of development and individual well-being of the tadpoles employed,—the main results obtained in respect of maintenance or non-maintenance of life and motion remain unaffected.

With regard to the nature of this sustaining power, we are at present prepared to do no more than put forward an explanation, which so far as it goes we believe to be correct, and which is borne out by further experiments to be quoted. Distilled water causes death by disintegration, primarily of the cuticle. By the addition of certain salts in the right proportion, we believe that the integrity of the tissues is maintained, and thus the life of the whole organism is preserved. The prevention of disintegration of tissue by the presence of minute quantities of some salts has been well shown in experiments referred to above on the ciliary action of gills of fresh water mussels. Portions of gill placed in distilled water rapidly changed; ciliary action ceased; the cells became granular and swollen, and separated from one another. In the presence of certain salts, however, ciliary action and structural integrity remained unimpaired for days.

Salts maintain life which to the heart of the frog are poisons,—for instance, the whole group of potassium salts, notably potassium oxalate and potassium cyanide. It cannot be supposed that a salt which paralyses the heart of the frog is inert when passing through the heart of the tadpole; we do not believe that these salts are absorbed when employed in such doses as we have found to sustain; we suppose them to be functional merely in maintaining the integrity of the skin and intestinal mucous membrane, thus protecting against themselves; since, with skin and mucous membrane intact both as regards structure and function, the physical laws of diffusion are held in abeyance, the cells exercising a selective power over what they absorb, or what they reject. Increase the dose, and the toxic action of the salt asserts itself; the selective function of the cells is paralysed, though in structure they remain intact; their controlling influence over the physical laws of diffusion is removed, and the salt is absorbed causing the death of the organism, although the structural integrity remains to all appearances unimpaired. This theory may be illustrated by the following experiment:—

Exp. 4.* *Potassium citrate* ($KH_2C_6H_5O_7$). In each beaker 200 c.c. distilled water; potassium citrate added so as to make percentage in

(1) ·00025 p.c.
(2) ·0005 „
(3) ·00075 „
(4) ·001 „
(5) ·00125 „
(6) ·0025 „
(7) ·005 „
(8) ·0075 „
(9) ·01 p.c.
(10) ·0125 „
(11) ·025 „
(12) ·05 „
(13) ·1 „
(14) ·15 „
(15) ·2 „
(16) ·25 „

April 25. 10 a.m. Tadpoles introduced.
2 p.m. (1) Two motionless. Two sluggish.
(2)—(16) All active (except a small one dead in 3).
26. 10 a.m. (1)—(3) All motionless.
(4)—(14) All active.
(15) and (16) All motionless.
27. 10 a.m. (4) Motionless.
(5) Two motionless. One active.
(6)—(12) Active (except one motionless in 10).
(13) Three active. One motionless.
(14) All motionless.
28. 10 a.m. (5)—(7) All motionless.
(8)—(12) All active (except one in 10).
(13) Three motionless. One active.

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* This experiment was performed in two parts on separate occasions; these have been arranged in a single series for the sake of clearness.

We believe that here the lowest doses of potassium citrate are not sufficient to obviate the distilled water action; the medium doses, at the same time as they suffice to sustain integrity, are not large enough to destroy the function of the cells, whereby diffusion is held in abeyance, and absorption of the salt prevented; the larger doses display the toxic action of the salt, paralysing the cells, allowing the physical laws of diffusion to act without check, so that the salt gains access to the tissues of the organism, and the tadpole dies.

Further evidence in favour of the non-absorption of salts in the doses in which they sustain is supplied by experiments with double solutions, containing potassium chloride and in addition one of various other salts. Of potassium chloride by itself, solutions have been tested varying in strength from 1 in 20,000 to 1 in 100; but only one (the weakest) has been found to maintain life so long as distilled water, none to maintain it longer (except in some cases of tadpoles in the gill stage, which present anomalous features). From experiments to be described, there is reason to believe that this lack of supporting power on the part of potassium chloride is due to its inability to entirely prevent the disintegrating action of distilled water; thus the salt gains access to the organism; and as would be expected the combined toxic action of the salt and disintegrating influence of distilled water cause death sooner than would distilled water alone. Disintegration must to some extent be obviated, since no gross changes occur comparable with those which are seen with distilled water alone; this however is no proof that minute breaches of integrity have not taken place, too small to be appreciated by the naked eye. If however a solution be employed, containing in addition to potassium chloride one or other of the salts which have been found to prevent disintegration, the result is very different; in such a double solution tadpoles survive for days, in many cases as well as in tap water, or in the sustaining solution itself free from potassium chloride.

We have tested in this respect solutions containing as well as potassium chloride one or other of the following salts,—potassium citrate, potassium oxalate, sodium oxalate, sodium phosphate, and calcium phosphate, and in every case the tadpoles have lived for days; in a solution containing potassium oxalate 1 in 4000 and potassium chloride 1 in 700 the tadpoles were alive and to all appearances quite healthy on the 8th day, when the experiment was closed. An experiment with potassium citrate + potassium chloride will be cited side by side with one in which potassium chloride was employed by itself.

Exp. 5. *Potassium chloride.* 200 c.c. distilled water in each beaker; to all but the first potassium chloride added to make percentage as follows:

(2) ·05 p.c.
(3) ·1 „
(4) ·15 „
(5) ·2 p.c.
(6) ·25 „

April 16. 11.30 a.m. Tadpoles introduced.
1 p.m. (1) Tadpoles still moving.
(2)—(6) All motionless.
4.30 p.m. All motionless.

Exp. 6. *Potassium chloride + potassium citrate* ($KH_2C_6H_5O_7$). In each beaker 200 c.c. of a ·01 p.c. solution of potassium citrate in distilled water; potassium chloride added so as to make percentage in

(1) ·1 p.c.
(2) ·15 „
(3) ·2 p.c.
(4) ·25 „

April 30.	10 a.m.	Tadpoles introduced.
May 3.	,,	All perfectly active.
4.	,,	All motionless.

It is seen that while the tadpoles in the potassium chloride solutions all died within 1½ hours, those in solutions containing corresponding quantities of potassium chloride, and in addition a small quantity of potassium citrate, survived for more than three days.

With a view of determining the nature of this controlling action of one salt over the toxic properties of another, the following pair of experiments was devised.

Exp. 7. *Potassium oxalate* ($K_2C_2O_4$) + *potassium chloride.* In each beaker 200 c.c. of a ·025 p.c. solution of potassium oxalate in distilled water; potassium chloride added so as to make percentage in

(1) ·15 p.c.
(2) ·25 ,,
(3) ·35 ,,
(4) ·5 ,,
(5) ·75 p.c.
(6) 1 ,,
(7) 1·25 ,,

April 29.	11 a.m.	Tadpoles introduced.
	2.30 p.m.	(1)—(6) All active.
		(7) All motionless.
30.	10 a.m.	(1)—(4) All active.
		(5) and (6) All motionless.
May 1.	,,	No change.
2.	,,	(1) and (2) Active.
		(3) and (4) One tadpole motionless in each.
3.	,,	(1) and (2) Active.
		(3) and (4) All motionless.
4.	,,	(1) All active.
		(2) Two active. One motionless.
5.	,,	(1) All active.
		(2) One only active.
6.	,,	No change.

Experiment discontinued.

By this experiment the dose was determined at which the toxic action of potassium chloride asserted itself despite the presence of a given quantity of potassium oxalate. A second experiment was then performed, in which this toxic dose of potassium chloride was employed as the constant, and to this solution increasing quantities of potassium oxalate were added.

Exp. 8. *Potassium oxalate + potassium chloride.* In each beaker 200 c.c. of a 1 p.c. solution of potassium chloride; potassium oxalate added so as to make percentage in

(1) ·025 p.c.
(2) ·05 „
(3) ·075 „
(4) ·1 p.c.
(5) ·125 „

April 30.	10.30 a.m.	Tadpoles introduced.
	1.30 p.m.	(1) All active.
		(2) Two active. One motionless.
		(3) Two active. One motionless.
		(4) One moving sluggishly. Two motionless.
		(5) All motionless.
	7 p.m.	(1) All active.
		(2) One active. Two motionless.
		(3) and (4) All motionless.
May 1.	10 a.m.	All motionless.

These two experiments show that there is no direct antagonistic action between the two salts; for had there been such action, the dose of potassium chloride which in Exp. 7 was toxic, would have been antagonised by the higher doses of potassium oxalate in Exp. 8; whereas in fact the higher the dose of potassium oxalate in the last experiment, the sooner the tadpoles died. We believe the interpretation of these experiments to be as follows. Potassium oxalate sustains the integrity of the superficial tissues of the tadpole, and thereby permits the presence of a certain quantity of potassium chloride in the solution with impunity to the tadpole, absorption not taking place. On increasing the strength of potassium chloride beyond a certain point, its toxic properties become evident in the paralysis of the functional activity of the skin and mucous membrane; although the line of cells remains unbroken, their powers of selection are in abeyance, and the physical law of diffusion comes into action, absorption of both salts taking place, and the organism rapidly becoming motionless. When once this limit is reached, it matters not, as shown by the experiments, whether potassium oxalate or potassium chloride be added: in either case the result is the same. These two salts therefore only differ in the extent to which they preserve integrity; when once access to the organism has been gained, the one is as fatal in its effect as the other.

The Influence of Saline Media on Tubifex Rivulorum. By SYDNEY RINGER, F.R.S., and ARTHUR G. PHEAR.

IN a communication shortly to be published in this *Journal* we have drawn attention to the influence of salts in sustaining the structural integrity of tadpoles. We now record the results of further investigations on the influence of saline media on tubifex rivulorum. As with tadpoles, so with tubifex, when placed in distilled water the animals in a few hours begin to disintegrate, and in 24 hours disintegration is complete, all structure being lost and only *débris* being left. Certain salts employed in appropriate strengths arrest or entirely obviate this disintegrating action of distilled water. Salts may protect against disintegration which are quite unable to sustain the functional activity of a frog's heart. Of all salts we have found those of calcium the most efficacious, notably the acid phosphate and the bicarbonate of calcium. Very small quantities of calcium salts suffice to maintain structure and life,—so minute as to produce merely a haziness with ammonium oxalate after standing for some minutes.

Calcium salts. The *acid phosphate* and the *bicarbonate* are the only two which sustain efficiently; the former is the better of the two. It was prepared by adding tribasic calcium phosphate to distilled water, and allowing the mixture to stand for 24 hours. The clear supernatant fluid was then removed, containing in solution a minute quantity of the acid phosphate of calcium, produced by the action of the carbonic acid present in the distilled water on the tribasic phosphate. This solution supports the integrity and life of tubifex as well as tap-water. *Calcium bicarbonate* solution was prepared by passing well washed carbonic acid through lime water until the milkiness first produced became almost dissolved. After standing for some time the clear overlying fluid was removed, and found to support life in tubifex when added to distilled water in the proportion of 60 c.c. to 140 c.c., and in stronger solutions. In this proportion the reaction of the solution was feebly alkaline.

Calcium hydrate, sulphate, nitrate, and *chloride* support life indifferently, a few organisms living three or four days. Of calcium hydrate, solutions only are efficient of neutral or slightly acid reaction; in these the lime is doubtless converted into the bicarbonate. The calcium sulphate solution was prepared by allowing distilled water to stand on sulphate of calcium for several days.

Sodium salts. Of *sodium hydrate* only very weak solutions (1 in 50,000 to 1 in 30,000) sustain, and these imperfectly; the neutral or faintly acid solutions are the best; alkaline solutions dissolve the organisms rapidly. *Sodium bicarbonate* sustains four or five days in solutions of 1 in 5000 to 1 in 2000. Neutral or faintly alkaline solutions are the most efficient. With larger quantities movement is arrested in 24 hours; but the shape and colour of the organisms are retained, and they recover completely if placed in tap-water. *Trisodium phosphate* solutions of neutral or faintly acid or alkaline reaction (1 in 4000 to 1 in 2000) sustain movement till the fourth day. In these doses structure is preserved after motion is arrested. Higher and more alkaline solutions cause a rapid dissolution of the animals. *Disodic phosphate* solutions of neutral or nearly neutral reaction (1 in 5000 to 1 in 2000) permit sluggish movement till the fifth day. Stronger solutions though arresting movement preserve integrity, and recovery ensues on placing the animals in tap-water. *Monosodic phosphate* solutions are very inefficient,—hardly better than distilled water; stronger solutions arrest movement, but obviate disintegration, and allow of recovery in tap-water. *Sodium chloride* though unable to sustain movement yet in strong solutions (1 in 2000 and stronger) keeps the organisms intact, and they recover when placed in tap-water. *Sodium nitrate* is quite inefficient.

Potassium salts. *Potassium hydrate.* Solutions of this in distilled water of neutral or almost neutral reaction support life imperfectly for four days. Alkaline solutions rapidly dissolve the animals. On the second day the solutions of potassium hydrate became acid, doubtless from absorption of carbonic acid, more than sufficient to convert the hydrate into the bicarbonate. *Potassium bicarbonate* is very inefficient; in a weak solution of faintly acid reaction sluggish movement persisted for three days; in strong solutions (1 in 280) the animals became motionless in 5 hours. *Tripotassium phosphate* solutions possess very imperfect sustaining powers; in neutral or faintly acid solutions slight movement was observed on the morning of the fourth day; in strong solutions the animals rapidly became dissolved. *Dipotassic phosphate* displays slight sustaining powers in neutral or nearly neutral solutions;

strong solutions cause rapid arrest of movement, but disintegration is avoided, and recovery takes place in tap-water. *Monopotassic phosphate* fails to maintain movement. *Potassium chloride* is quite inefficient to support movements. In the weaker solutions the animals disintegrate; in higher solutions integrity is preserved, and movement is restored readily by tap-water.

Certain salts then obviate disintegration. Of these some, as will be shown, are of highly poisonous nature. Of many of these poisonous salts however the percentage requisite to maintain integrity is so small as to be probably quite innocuous. Other poisonous salts preserve integrity and movement in comparatively large doses; such are the oxalates of sodium and potassium. Of these salts we do not suppose absorption to occur. With intact and living skin or mucous membrane the physical laws of osmosis are held in abeyance, and a selective function is possessed and exercised by the live tissue. If a sufficiently strong solution of any salt be employed, this selective function is paralysed, osmosis occurs, the salt gains access to the organism, which becomes motionless and in a short time dies.

The nature of this protective influence over integrity is obscure. In many of our experiments it appears that solutions of neutral or nearly neutral reaction are the most efficient. Reaction however though important is not the sole factor. Some salts, of themselves neutral, and therefore leaving unmodified the acidity of distilled water powerfully protect. Such salts are the magnetic oxide of iron and potassium ferrocyanide. Moreover calcium salts are extremely competent to sustain, to a great extent, independently of their reaction. Distinctly acid solutions of calcium phosphate, and strongly alkaline solutions of calcium bicarbonate sustain as well as tap-water.

Again, it may be asked:—in the maintenance of integrity and movement, do salts act as a whole, or by virtue of one or other of their constituents? Although we cannot claim to have settled this question, yet some experiments devised to elucidate the matter may be of sufficient interest to be recorded. Carbonic acid is a common constituent of calcium, potassium and sodium bicarbonate, and occurs also in distilled water. Yet the last is incapable of supporting integrity; and the first three though all efficient in this respect yet vary widely in their degree of efficiency. Moreover tap-water saturated with carbonic acid gas acts in a very different manner on tubifex from distilled water similarly treated. Tubifex when introduced into either become almost immediately motionless; movement however rapidly returns to those placed in tap-water, and in a few hours they are seen to be again perfectly active. Those in distilled water on the other hand remain motionless, and while retaining their shape in the course of twenty-four hours become opaque and granular looking—a peculiar appearance which we have learnt to associate with the action of acids.

The action of *potassium ferrocyanide* may here be described. This salt is very efficient to maintain, in solutions varying from 1 in 400,000 to 1 in 28,000; the lowest of these permitted a small amount of disintegration: but in all the animals were active in their movements on the fifth day. The solutions were slightly acid, all to the same degree, the acidity being that of the distilled water employed. Stronger solutions while maintaining integrity hampered and even arrested movement; complete recovery ensued on placing the animals in tap-water. *Sodium ferrocyanide* acts similarly to the potassium salt. *Potassium ferricyanide* resembles the ferrocyanide in supporting life and movement well. Of the constituents of potassium ferrocyanide, *potassium* has been shown to sustain movement imperfectly in the form of the hydrate, bicarbonate and phosphate. *Iron* in the form of the magnetic oxide perfectly supports life and movement even in minute quantities. Pure magnetic oxide of iron free from lime (which is often present in commercial magnetic oxide) was shaken up with distilled water and allowed to stand for five days. The clear supernatant fluid was removed and found to contain about 1 part of Fe_2O_3 to 500,000[1]. This minute quantity is very efficient in sustaining integrity and motion. It might be supposed that the iron acted by combining with the carbonic acid in the distilled water, on the assumption that it is the carbonic acid that destroys. This is disproved by the fact that the iron water was quite as acid as distilled water, and yet this solution sustained thoroughly well. *Potassium cyanide* imperfectly supports movement for three or four days in doses from 1 in 150,000 to 1 in 28,000. Stronger solutions with marked alkaline reaction quickly dissolve the tubifex. *Hydrocyanic acid* even in very minute quantities (1 in 2,000,000) sustains movement some hours longer than distilled water. Stronger solutions are no better than the weaker ones. It appears then that all the constituent parts of potassium ferrocyanide support integrity and life, although iron is the most effectual.

In the case of *oxalates* the acid constituent appears to play a more important part than the sodium or potassium. *Sodium oxalate* in solutions of 1 in 20,000 to 1 in 2000 preserved activity for more than four days. Higher strengths rapidly arrested movement, but preserved integrity, and the addition of calcium chloride to the solution was followed by recovery. In solutions of *neutral potassium oxalate* (1 in 25,000 to 1 in 4000) movement continued till the third day. *Potassium binoxalate* in weak solutions sustained languid movement to the third day. *Oxalic acid* in minute quantities proved very efficient. Solutions containing 1 in 200,000 to 1 in 50,000 sustained well; on the fifth day the animals were clustered and active. Larger quantities speedily kill, but the animals retain their shape, becoming of a dull opaque white colour. No sustaining properties have been discovered in *phosphoric acid.* It seems then that the constituent concerned in maintaining integrity varies with different salts, in some cases being the acid element, in others the base. With calcium phosphate and calcium bicarbonate and the corresponding salts of sodium and potassium, the preservation of integrity appears to be due to the calcium, sodium and potassium respectively; whilst with sodium oxalate and potassium oxalate the oxalic acid is the more potent constituent.

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[1] The analysis was made by Mr B. Moore, in the Physiological Laboratory of University College.

Brief reference must be made to the property displayed by calcium salts, oxalates and iron in protecting tubifex (and tadpoles) from the poisonous effects of other salts. The presence of calcium phosphate or bicarbonate in solutions of potassium chloride which by themselves would arrest movement in about three hours entirely obviates this paralysing action of potassium chloride. The oxalates of sodium and potassium and the magnetic oxide of iron possess similar properties, but to a slighter degree. These salts permit movement to continue for some days in a solution of potassium chloride; with lime salts under similar circumstances movement persists indefinitely. But calcium salts, as we have seen, antagonise the action of potassium salts in another way. Tubifex which have been rendered motionless by potassium chloride, on the addition of calcium chloride completely recover. The paralysed animals retain their shape and colour, and it must be supposed that the potassium salt is absorbed and arrests function by its direct action on the tissues of the organism. Revival in these cases must be due to an antagonism, effected in the tissues, on the part of the lime salt over the paralysing action of the potassium chloride. Magnetic oxide of iron and oxalate of sodium or of potassium are powerless in this respect; they appear to act solely by preserving integrity and preventing absorption, and are unable to antagonise the action of potassium chloride on the tissues.

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FURTHER OBSERVATIONS REGARDING THE ANTAGONISM BETWEEN CALCIUM SALTS AND SODIUM POTASSIUM AND AMMONIUM SALTS.

By SYDNEY RINGER, M.D., F.R.S.

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IN previous communications I have drawn attention to the mutual antagonism between calcium and potassium salts on the frog's heart[1] and their antagonism on the entire organism in the case of tubifex[2], also to the antagonism between calcium salts on the one hand and sodium and potassium salts on the other[1] on the clotting of blood. In this paper I continue the subject and shall show that an antagonism exists between calcium salts and sodium and potassium and ammonium salts as regards their influence on muscular contraction (frog's heart) and on the clotting of milk by rennet.

In view of Dr Locke's recent important and interesting investigations[3], I may state that the distilled water I have used and experimented with was in all cases obtained from a distilling apparatus made of metal.

Influence on clotting of Milk.

In these experiments the milk was diluted with three parts of distilled water. This mixture (with most specimens of milk) with rennet[4] and heated to 50° C. will not clot. The calcium salts are so diluted that they are unable to cause clotting (precipitation of casein), which however readily occurs on adding a few drops of a 10 % solution of calcium chloride. In this respect the clotting of milk is similar to the clotting of blood and the contraction of the heart muscle. If blood be sufficiently diluted it clots very slowly or not at all, but a clot can be speedily produced by adding a calcium salt like calcium chloride[1].

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[1] This *Journal*, XI. 369. 1890.

[2] This *Journal*, XVI. 1. 1894.

[3] This *Journal*, XVIII. 319. 1895.

[4] The rennet was prepared by Martindale and was free from calcium salts. A 10 % $CaCl_2$ solution was employed.

Five test-tubes each received 15 c.c. of diluted milk, 1 in 4. To 2, 3, 4 and 5 were added respectively 5, 10, 15 and 20 drops of 10% solution of sodium chloride and to 1, 2, 3 and 4, 20, 15, 10 and 5 drops of distilled water. Then to each solution was added five drops of rennet and the tubes were placed in a bath of 50° C. After 20 minutes the bath was heated to boiling point, but none of the specimens clotted. The tubes were then cooled and I added to each six drops of 10% $CaCl_2$ solution but no clotting occurred at the temperature of the room. The test-tubes were then placed in a bath of 50°C. In about six minutes the fluid in 1 became granular, the milk in the other tubes (*i.e.* those containing NaCl solution) remained unchanged. Next morning, twenty hours later, the first tube contained a contracted clot. The rest, *i.e.* those containing NaCl solution, were unclotted.

Another six drops of calcium chloride were added to each of these tubes and they were placed in a bath of 50°C. for ten minutes, none of the milk clotted. Next morning, about twenty hours later, these tubes contained no clot but only a slight granular deposit decreasing in amount with the increase in the quantity of sodium chloride.

In this experiment the tube which received no sodium chloride clotted with six drops of calcium chloride solution in about five minutes, whilst this quantity of calcium chloride was unable to clot the milk in the tubes containing five drops and upwards of sodium chloride. An addition of six drops of calcium chloride with the aid of a temperature of 50°C. was only able slowly to cause a slight granular deposit most marked in those tubes containing least sodium chloride.

Similar experiments were made with potassium chloride. Placed in a bath of 50°C. in a quarter of an hour none were clotted. The tubes were then cooled, six drops of calcium chloride solution were added to each tube and the tubes were again heated to 50°C. In two minutes the tube, without potassium chloride solution, and those containing five and ten drops respectively clotted, and in seven minutes the milk containing fifteen drops of potassium chloride solution also clotted. After three hours the fluids remained in the same state except that the clot in the milk containing fifteen drops of potassium chloride solution was small and not so firm as in the tubes containing less potassium chloride. Six drops of calcium chloride solution were added to the tube containing twenty drops of potassium chloride solution and the tube was placed in a bath of 50° C. The milk speedily clotted. This experiment therefore shows that there is a well-marked antagonism between potassium chloride and calcium chloride, but potassium chloride antagonises calcium chloride much less than sodium chloride.

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[1] This *Journal*, XI. 369.

Similar experiments to the preceding were made with ammonium chloride. The tubes were placed in a bath of 50° C., in fifteen minutes none of the specimens were clotted, six drops of calcium chloride solution were added to each tube after the fluid had been cooled and the tubes were again placed in a bath of 50°. In five minutes the milk in the three first tubes, *i.e.*, that without ammonium chloride and those with 5 and 10 drops respectively, were clotted, after an interval of 30 minutes, the fluids having been cooled, three drops of calcium chloride solution were added to tubes 5 and 6, and these were placed in a bath of 50°, but this did not cause clotting. After two hours another three drops of calcium chloride solution were added and the tubes placed in a bath of 5°, the milk in both speedily clotted.

In clotting of milk by rennet two distinct processes occur which can be dissociated[1]. Rennet converts caseinogen into casein (gerinnung) which remains in solution unless enough calcium salt is present to produce clotting (abscheidung). For if caseinogen is precipitated by acetic acid and the precipitate is well washed and then rubbed in a mortar with calcium carbonate, on the addition of distilled water the precipitate dissolves into a milky solution. On adding rennet to some of this and heating to 40 or 50° C., no clotting occurs, but the chemical change (gerinnung) from caseinogen to casein has been induced, for if the solution be boiled and the rennet thus destroyed, and the solution cooled, on the addition of a calcium salt, such as calcium chloride, and heating to 30° or 40° C. a clot speedily forms. The rennet produces the chemical change by which caseinogen becomes converted into casein, but this remains in solution till on the addition of calcium chloride there is first produced a granular condition of the fluid, and then these granules unite to form a well-marked clot.

The antagonism between calcium salts and sodium, potassium and ammonium salts is limited to the precipitation of the casein as a clot and does not affect the chemical change from caseinogen to casein. This is shown in the previous experiments where, after heating to 50°

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[1] This *Journal*, XII. 164.

for some time, the various fluids were boiled and so the rennet destroyed, yet after cooling and adding calcium chloride and warming the solution to 40° C., a well-marked clot occurred. The rennet converted caseinogen into casein, but this remained in solution until sufficient calcium salt was added to antagonise the sodium or potassium or ammonium chloride when a clot was formed.

Antagonism on the Frog's heart.

I have already shown that calcium and potassium salts are mutually antagonistic in respect of their action in the frog's heart. If the frog's heart is supplied with a suitable circulating fluid such as calcium triphosphate and potassium chloride, and then a solution of potassium is added the beats speedily grow weaker and at last contractility ceases, but is completely restored on adding a sufficient quantity of calcium chloride.

A similar antagonism exists between sodium chloride and calcium chloride and between ammonium chloride and calcium chloride.

I perfused the ventricle with 200 c.c. of phosphate of calcium circulating fluid and then added successive quantities of 10 $^0/_0$ solution of sodium chloride. On reaching 16 c.c. of sodium chloride solution the contraction had become very weak, but the contractions completely recovered on the addition of 4 c.c. 1 $^0/_0$ solution of calcium chloride.

Calcium chloride also antagonises the action of ammonium chloride, but the antagonism is not so pronounced as with potassium chloride and sodium chloride.

The following experiments show that there is a well-marked antagonism between calcium bicarbonate[1] and sodium chloride and between calcium bicarbonate and ammonium chloride, on tubifex[2].

A series of experiments were made. Seven beakers each received 200 c.c. of a solution of calcium bicarbonate[1] and six beakers received 200 c.c. of distilled water. Into the second to the seventh and into the eighth to the thirteenth beaker was added in an ascending scale from 5 c.c. to 10 c.c. of 10 $^0/_0$ solution of sodium chloride. We thus tested the antagonism between calcium bicarbonate and sodium chloride and had control experiments with sodium chloride alone. In five hours the tubifex in sodium chloride solution were quite motionless, whilst those in the first beaker containing calcium bicarbonate solution alone, and the second to the seventh beaker those containing calcium bicarbonate solution with different quantities of sodium chloride, were quite active on the fifth day.

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[1] Made by passing carbonic acid through equal parts of lime water and distilled water and then filtering.

[2] Calcium salts similarly antagonise potassium salts. See This *Journal*, XVI. 1.

In another experiment we increased the quantity of sodium chloride to 12, 14, 16 and 18 c.c. respectively, and on the fifth day the tubifex were quite natural.

As with potassium chloride so with sodium chloride, the arrest of movement with these large quantities of sodium chloride is not due to disintegration of the animal, for the animals remain entire, and even after two days of complete paralysis, if placed in tap water, quite recovered, hence the arrest of movement is due to the action of sodium chloride and potassium chloride on the tissues after absorption and this action is antagonised by calcium bicarbonate.

Similar experiments were made with ammonium chloride. A distinct antagonism occurred, but it was much more limited than with sodium chloride, for with this the calcium bicarbonate solution antagonised a solution containing one part of sodium chloride to 120 parts of calcium bicarbonate solution and probably could have antagonised sodium chloride in stronger solutions, but none stronger were tested. With ammonium chloride, the calcium bicarbonate solution only antagonised one part of ammonium chloride in 1000 parts of calcium bicarbonate solution.

In these experiments I find that in the clotting of milk, sodium chloride antagonises calcium chloride more powerfully than potassium chloride or ammonium chloride, whilst as regards the clotting of blood, sodium chloride is only a little more active than potassium chloride in its antagonism to calcium chloride, whilst as regards cardiac muscular contractility potassium chloride antagonises calcium chloride far more powerfully than sodium chloride.

When one bears in mind how similar the three processes, muscular contraction, coagulation of blood, and clotting of milk are in many respects, and as it has been shown that the clotting of milk consists of at least two distinct and separable processes and that calcium salts influences only one of these, it is I think justifiable to suggest the probability that clotting of blood and muscular contractility are dependent on several changes and that calcium salts only affects one of these.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

December 11*th*, 1897.

ST THOMAS'S HOSPITAL.

The action of Distilled Water on Tubifex. By SYDNEY RINGER, M.D., F.R.S.

Soon after Mr Locke published his important communication[1], showing the poisonous and disintegrating action of heavy metals such as copper, even in infinitesimal quantities on tubifex and tadpoles, I repeated many of his experiments and entirely confirm his results.

I now publish a few more observations bearing on this subject and which also confirm his conclusions. I evaporated in large quantities distilled water similar to that I had previously used and obtained from the Chemical Laboratory of University College. The products of the first evaporation were rejected and the retort washed out. Thirty litres were then evaporated and the distillate used in the experiments. The fluid residue in the retort had an abundant whitish deposit and a strong alkaline reaction. Tested, it gave undoubted reactions of copper.

After testing the effect of the distilled water on tubifex, I then tried the action of the residue in the retort. Tubifex placed in this fluid quickly disintegrated, but when this fluid stood exposed to the air for several days, till the alkalinity was only slight or lost then tubifex lived as well as in tap water. I only record this experiment without giving any explanation beyond surmizing that by the exposure the copper was precipitated and so became innocuous.

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[1] *Journ. Physiol.* XVIII. p. 319. 1895.

For the further elucidation of this question, I collected rain water in earthen glazed and glass vessels. I first collected the rain water on the roof of University College. This water gave a strong acid reaction and quickly disintegrated tubifex but on adding a very small quantity of sodium bicarbonate so as to neutralize the acidity, the water well sustained the integrity and life of tubifex. I next collected rain water in a field adjoining an extensive Moor in Yorkshire far away from any houses. This water was not quite clear. No doubt it was mixed with atmospheric dust. It was neutral in reaction and free from any calcium salt. It sustained the life of tubifex as well as tap water. To some of this rain water a few small pieces of copper filing were added, and the water was shaken several times a day for four days and then the water was poured off the copper filings. Tubifex placed in this water were disintegrated in six hours. I next tested with this rain water from the Moors the antagonism between potassium chloride and calcium chloride.

I placed tubifex in a ·1% solution of potassium chloride, in about four hours they were almost motionless and with the microscope I was unable to see the vessels contract; they appeared quite paralysed except just below the head of the animals. I then placed the tubifex in a ·1% KCl and ·1% CaCl^2 and almost at once spontaneous movement increased, and in five minutes they became quite active. Seven hours later they were moving energetically, before movement was so much restored the microscope showed that the vessels even to the tail were contracting vigorously.

I next used ·25% solutions of KCl and CaCl^2 repeating the above experiment and with similar results.

These experiments appear to me to establish Locke's conclusion that copper in even infinitesimal quantities will disintegrate tubifex whilst water free from copper or other heavy metals and without any salts such as calcium salts can sustain the life of tubifex.

Papers and References in "The Lancet."

YEAR	VOL.	PAGE	
1862	I	173	On the Temperature, Urea, Chloride of Sodium, and Urinary Water in Scarlet Fever; and On a Cycle in Disease and Health.
1864	II	675	Testimonial to Dr. Sydney Ringer, of University College Hospital.
1865	II	429	Review. "The Temperature of the Body as a means of Diagnosis in Phthisis and Tuberculosis."
1865	II	512	University College Hospital: The Thermometer in Acute Disease: Case of Renal Inflammation following Scarlatina; Clinical Remarks.
1865	II	648	University College Hospital: The Thermometer in Specific Fever: Case of Typhoid Fever; Remarks.
1866	II	208	The Influence of Alcohol on the Temperature of Non-Febrile and Febrile Persons.
1866	II	381	University College: Dr. Sydney Ringer's Address.
1869	I	42	Paper No. 1 on The Therapeutic Action of Drugs: Aconite and its Preparations.
1869	I	392	Paper No 2, ditto: Bromide of Potassium; Bromide of Sodium; Bromide of Ammonium.
1869	II	10	Review. Dr. Ringer's Handbook of Therapeutics.
1871	II	752	Ditto.
1873	I	53	Ditto.
1873	I	877	Review. "The Temperature of the Body as a means of Diagnosis and Prognosis in Phthisis."
1873	II	469	Letter by Dr. Ringer on the Accidental Poisoning of Dr. Sharpey; Recovery.
1874	I	264	On Sulphide of Potassium, Sulphide of Sodium, and Sulphide of Calcium.

YEAR	VOL.	PAGE	
1874	II	338	Ipecacuanha Spray in Winter Cough and Bronchitic Asthma.
1874	II	805	Review. A Handbook of Therapeutics.
1875	I	157	On Jaborandi. By Dr. Ringer and Dr. Alfred P. Gould.
1875	II	907	On Gelseminum Sempervirens. By Dr. Ringer and Wm. Murrell, L.R.C.P., M.R.C.S.
1876	I	82	Ditto.
1876	I	124	Ditto. The Influence of Gelseminum on the Frog's Heart.
1876	I	415	Ditto. The Influence of Gelseminum on Respiration.
1876	I	561	Ditto. Ditto.
1876	I	661	Ditto. Action on Man.
1876	I	732	Ditto. Influence on the Eyes.
1876	I	921	Royal Medical and Chirurgical Society: An Abstract of the following Paper by Dr. Ringer was read: "Observations on Box (Buxus Sempervirens), with special reference to the true nature of Tetanus."
1876	II	78	On Gelseminum Sempervirens. Action on the Pupils.
1876	II	569	Ditto. Action on the Circulation.
1877	I	228	A Suggestion concerning the Condition of the Nervous Centres in Migraine, Epilepsy, and other Explosive Neuroses.
1877	I	499	Review. A Handbook of Therapeutics.
1877	II	198	The Action of Muscarin on the Human Body. By Dr. Ringer and E. A. Morshead, M.R.C.S., L.R.C.P.
1877	II	912	On the Use of Physostigma in some Nervous Affections. By Dr. Ringer and Wm. Murrell, M.R.C.P.
1877	II	950	Ditto.
1878	I	858	On Gelseminum Sempervirens. Gelseminum as a Toxicological Agent.
1878	I	892	On Gelseminum Sempervirens.
1878	II	473	Concerning the Influence of Perspiration on the Febrile Temperature.
1879	I	160	Review. A Handbook of Therapeutics.
1879	I	290	The Action of Pituri on Man.
1880	I	360	On Tonga: A Remedy for Neuralgia, used by the Natives of the Fiji Islands. By Dr. Ringer and Wm. Murrell, M.D., M.R.C.P.

YEAR	VOL.	PAGE	
1880	I	407	Review. A Handbook of Therapeutics.
1880	I	795	On the Mydriatic Properties of Homatropin, with an Account of its General Physiological Action. By John Tweedy, F.R.C.S., and Sidney Ringer, M.D.
1880	II	6	On Glycerine in Flatulence, Acidity, and Pyrosis. By Dr. Ringer and Wm. Murrell, M.D.
1882	I	1033	Royal Medical and Chirurgical Society: Abstract from a Paper by Dr. Ringer and Dr. Harrington Sainsbury concerning the Action of Salts of Potash, Soda, and Ammonia on the Frog's Heart.
1882	II	12	Review. A Handbook of Therapeutics.
1882	II	736	On the Action of the Salts of Sodium, Ammonium, and Potassium. By Dr. Ringer and Dr. Harrington Sainsbury.
1883	I	7	On Manganese in the Treatment of Amenorrhœa. By Dr. Ringer and Dr. Wm. Murrell, M.R.C.P.
1883	I	87	Letter by "Sceptic" criticizing The Paper on Manganese in the Treatment of Amenorrhœa, by Dr. Ringer and Dr. Murrell.
1883	I	628	Concerning the Effects of Dilution and Concentration on the Actions of Poisons.
1883	II	766	Article on Nitrite of Sodium as a Toxic Agent. By Dr. Sydney Ringer and Dr. Wm. Murrell.
1883	II	880	Letter by Dr. Ringer in reply to Dr. Murrell's explanatory Letter on "Nitrite of Sodium as a Toxic Agent."
1883	II	996	Review. A Handbook of Therapeutics.
1884	II	767	Article on the Nervous or Muscular Origin of certain Spastic Conditions of the Voluntary Muscles. By Dr. Sydney Ringer and Dr. Harrington Sainsbury.
1884	II	815	Ditto.
1884	II	860	Ditto. Conclusion.
1885	I	6	Article on an Examination of the Supposed Action exerted by the Antagonism between Acids and Alkalies in modifying the Tonicity of the Heart and Arteries. By Dr. Sydney Ringer and Dr. Dudley Buxton.
1886	I	792	Review. A Handbook of Therapeutics.
1888	II	1082	Annotation. "Ringer's Therapeutics: Strange Advice."

YEAR	VOL.	PAGE	
1889	I	227	Review. A Handbook of Therapeutics.
1891	II	1212	Article on Pulsations and Murmurs in the Great Veins of the Neck ; their Physiological and Clinical Significance. By Dr. Ringer and Dr. Harrington Sainsbury.
1891	II	1268	Ditto.
1892	I	740	Ditto.
1892	I	790	Ditto. Conclusion.
1894	I	325	Article on "Some Practical Points concerning the Diagnosis of Cardiac Murmurs." By Dr. Sydney Ringer and Dr. Arthur Phear.
1894	II	482	Review. A Handbook of Therapeutics.
1894	II	729	Article on "The Clinical Significance of Accentuated Second Sound." By Dr. Ringer and Dr. Arthur G. Phear.
1896	I	166	Clinical Society of London : Dr. Ringer and Dr. Arthur Phear contributed an Account of a Case of Addison's Disease treated with Suprarenal Extract.
1905	I	582	Reference to Dr. Ringer in a leading article on "Shifting Dullness in the Thorax and Abdomen."
1910	II	1386	Obituary Notice.

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YEAR	VOL.	PAGE	
1859	—	680	Royal Medical and Chirurgical Society: An Abstract of a Paper by Dr. Ringer on "The Connection between the Heat of the Body and Excreted Amounts of Urea, Chloride of Sodium and the Urinary Water, during a Fit of Ague."
1860	—	654	Royal Medical and Chirurgical Society: Paper by Dr. Ringer on "The Relative Amount of Sugar and Urea in the Urine of Diabetes Mellitus." Communicated by Mr. Richard Quain, F.R.S.
1861	I	343	Note *re* a Paper by Dr. Ringer, in the Edinburgh Medical Journal of February, 1861.
1862	I	211	Royal Medical and Chirurgical Society: Paper by Dr. Ringer on "The Temperature, Urea, Chloride of Sodium and Urinary Water in Scarlet Fever and on a Cycle in Disease and Health." Communicated by A. B. Garrod, M.D., F.R.S.
1865	I	248	Review. "On the Temperature of the Body as a Means of Diagnosis in Phthisis and Tuberculosis." By Dr. Ringer.
1866	II	289	Royal Medical and Chirurgical Society: "The Influence of Alcohol on the Temperature of Non-Febrile and Febrile Persons. By Dr. Ringer and Walter Rickards, M.D.
1869	II	269	Review. "A Handbook of Therapeutics." By Dr. Ringer.
1874	II	288	On the Action of Croton-Chloral Hydrate in Certain Forms of Megrim. (Abstract: Proceedings of Section of Medicine, Annual Meeting, British Medical Association, Norwich).
1874	II	637	Paper in full.
1875	I	380	On the Value of Tar in Bronchial Catarrh and Winter Cough. By Dr. Ringer and W. M. Murrell, L.R.C.P., M.R.C.S.

YEAR	VOL.	PAGE	
1875	I	543	"On Jaborandi."
1875	II	425	A Case of Rheumatic Hyperpyrexia cured by one Cold Bath.
1875	II	775	"A Case of Diabetes Insipidus treated by (1) Jaborandi and (2) Ergot."
1881	I	159	University College Hospital: "A Case of Delirium in Acute Rheumatism under the care of Dr. Ringer. Reported by Mr. David Collingwood.
1882	I	539	University College Hospital: "A Case of Epithelioma of Œsophagus under the care of Dr. Ringer." Reported by Mr. S. H. Collins.
1882	I	943	Royal Medical and Chirurgical Society: "The Action of Salts of Potash, Soda and Ammonia on the Frog's Heart." By Dr. Ringer and Dr. Harrington Sainsbury.
1882	II	1134	"Action of the Element Arsenic." By Dr. Ringer and Dr. Harrington Sainsbury.
1882	II	1193	Continued.
1883	I	451	"An Experimental Investigation of the Action of Chloral, Opium and Bromide of Potassium." By Dr. Ringer and Dr. Harrington Sainsbury.
1883	I	556	Continued.
1883	II	265	"Investigations into the Physiological Action of Barium Chloride." By Dr. Ringer and Dr. Harrington Sainsbury.
1883	II	925	Review. "A Handbook of Therapeutics." By Dr. Ringer.
1883	II	1070	Royal Medical and Chirurgical Society: "Investigations into the Action of the Digitalis Group."
1884	I	97	"Note on some Experiments with Ergotine." By Dr. Ringer and Dr. Harrington Sainsbury.
1884	II	114	Report on the Influence of Rhombic Sodium Phosphate and Sodium Bicarbonate on Muscular Contraction.
1885	I	731	Regarding the Effect of the Saline Ingredients of the Blood on the Contractions of the Heart.
1886	I	303	Review. "A Handbook of Therapeutics." By Dr. Ringer.

YEAR	VOL.	PAGE	
1887	II	1033	Address to the Midland Medical Society: "The Antagonism of Drugs."
1888	I	1257	"Remarks on Paroxysmal Sneezing." By Dr. Ringer and Dr. Murrell. Part I.
1888	I	1327	Part II.
1889	I	139	Review. "A Handbook of Therapeutics." By Dr. Ringer.
1894	II	1499	University College Hospital: Notes on the Antitoxin Treatment of Diphtheria, under the care of Dr. Ringer. Reported by Mr. F. Travers.
1895	II	1412	"Preparation of Milk for Diabetic Patients."
1895	II	1524	Letter by Dr. Ringer on "Preparation of Milk for Diabetic Patients."
1896	I	150	Clinical Society of London: "Addison's Disease treated with Suprarenal Extract." By Dr. Ringer and Dr. Arthur Phear.
1896	II	195	University College Hospital: Case of Antifebrin Poisoning, under the care of Dr. Ringer. Reported by Dr. Alfred Dimsey.
1896	II	1543	Letter by Dr. Ringer on "Belladonna in Bronchitis."
1898	I	701	Review. "A Handbook of Therapeutics." By Dr. Ringer.
1901	I	398	Royal Medical and Chirurgical Society: Remarks by Dr. Ringer during a discussion on "The Outbreaks of Arsenical Poisoning in Beer Drinkers."
1910	II	1276	Formal Note announcing the Death of Dr. Ringer.
1910	II	1384	Obituary Notice.

15/10/85